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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

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STEM ROT: A DISEASE OF COWPEAS CAUSED BY AN UNDESCRIBED SPECIES OF *PHYTOPHTHORA*

By G. S. PURSS, B.Agr.Sc., Pathologist, Science Branch, Division of Plant Industry.

SUMMARY.

A stem rot disease of cowpeas which is the cause of serious losses in Queensland is described.

A species of Phytophthora **is shown to be the causal agent and is described in detail. The name** Phytophthora vignae **n. sp. is proposed.**

Field observations show that the disease is soil-borne and that there is a build-up of the pathogen in the soil as a result of growing successive crops of susceptible varieties.

Sporangia formed on disease lesions are capable of producing infection above ground level. It is suggested that this may explain the rapid spread of the disease in moist weather.

Water plays an important role in the dissemination of the disease. Excessive water increases the incidence of disease on lightly infested soil but on heavily contaminated soil a high moisture level is not required for serious disease development.

It has not been possible to demonstrate seed-borne infection. No control of the disease was obtained by fungicidal treatment of seed prior to sowing in infested soil.

Sowing time has a marked influence on disease incidence, there being a fall of infection with falling temperatures under glasshouse conditions. The range of temperature over which infection can occur in the laboratory (66–82 deg. F.) is narrower than the range over which growth of the fungus can occur (52–93 deg. F.).

I. INTRODUCTION.

A stem rot condition in cowpeas was first recorded in Queensland early in 1952 (Purss 1953). In that and successive years severe outbreaks of the disease were recorded throughout most of the State. Poona and Reeves, the principal varieties grown, have both proved particularly susceptible to the disease and many growers are now refraining from planting the crop on this account.

A wilt of cowpeas caused by a species of *Phytophthora* has also been recorded from Carlingford in New South Wales (New South Wales Department of Agriculture 1950), and a similar disease was later responsible for heavy losses at Kyogle (New South Wales Department of Agriculture 1955). It is suspected that this disease is identical with the one described in this paper, as isolations from material recently supplied from New South Wales yielded a *Phytophthora* identical with the one occurring in Queensland.

A disease described as red stem canker has been reported in the United States of America (Weimer 1949). The causal organism was shown to be *Phytophthora cactorum* (Leb. & Cohn.) Schroet. The symptoms described are somewhat similar to those of the Queensland disease, with minor differences. However, the organism responsible in this State is distinct from that described by Weimer.

II. SYMPTOMS.

When the disease occurs in an area where it has never previously been encountered it is usual for the outbreak to be confined at first to isolated patches. These patches rapidly expand during favourable weather conditions. On the other hand, where the disease has been reported during previous seasons it is usual for the first infected plants to be scattered throughout most of the paddock.

Affected plants turn yellow; during moist weather they may survive for a considerable time but in dry conditions they rapidly wilt and die (Fig. 1).

Fig. 1.

A Cowpea Crop of the Poona Variety Affected with Stem Rot.

Fields varying in size from 2 acres to 30 acres in which it has been difficult to find healthy plants have been observed. The disease has been located under a variety of conditions of soil type and drainage from black alluvial flats to poor stony hillside country. Plants in all stages of growth from seedling to maturity are subject to attack. Wilting is very rapid in infected seedlings.

Fig. 2.

Lesion Development on Diseased Plants.

Individual plants are characterised by a brown lesion commencing at ground level and extending upwards, often reaching the top of the stem. This lesion is sunken and completely girdles the stem. The margin is usually watersoaked and during moist weather is covered by a light fungal growth in which sporangia can be distinguished. Internally, a brown discoloration extends for a short distance into the healthy tissue. The root system rapidly decays and is usually covered by a mat of white and pink fungal spores (Fig. 2).

In addition to these typical symptoms, under moist conditions it is common to find aerial lesions high up on an otherwise healthy stem (Fig. 3).

The red coloration characteristic of red stem canker is not apparent with this disease.

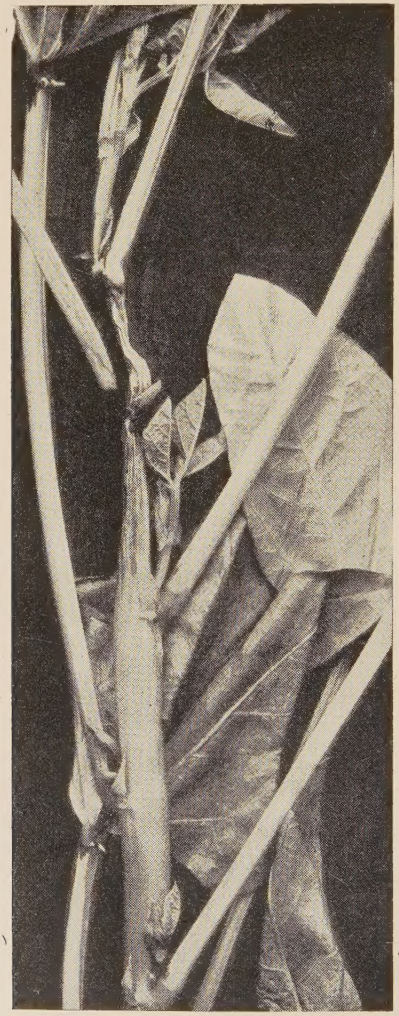

Fig. 3.

Stem Decay Resulting from Aerial Infection.

III. ETIOLOGY.

(1) Isolations.

Many fungi have been isolated from diseased lesions (both basal and aerial) but those that appear regularly are *Fusarium* spp., *Macrophomina phaseoli* (Maubl.) Ashby and a white phycomycete. Fruiting bodies of the first two fungi are usually abundant on the earlier affected parts. The white phycomycete can only be isolated from the advancing edge of the lesion.

(2) Pathogenicity Tests.

The species of *Fusarium* and *M. phaseoli* were inoculated on to healthy cowpea seedlings of the variety Poona by dipping the roots into a slurry formed by macerating in water a potato dextrose agar (P.D.A.) culture of the fungus being tested. No infection was obtained in either case.

The white phycomycete was tested by the method described by Weimer (1949). Cowpea seedlings of the variety Poona approximately four weeks old were raised in pots of sterilized soil. These were inoculated by placing small pieces ($\frac{1}{4}$ in. square) of a P.D.A. culture of the fungus on the uninjured stem tissue just above ground level. These pots were then well watered and covered with a bell-jar. A similar series of inoculations was done in which sterile agar was used as inoculum.

Seedlings inoculated with the phycomycete commenced to show lesion development at ground level in three days and at 10 days all seedlings had collapsed. Seedlings inoculated with sterile agar remained healthy.

Results of a typical series of pathogenicity tests are given in Table 1. These tests were repeated a number of times and on each occasion gave similar results.

Table 1.

RESULTS OF PATHOGENICITY TESTS ON COWPEAS.

Treatment.	Number of Plants Treated.	Number Affected After 10 Days.
(1) Inoculated above ground with phycomycete. Moist chamber	12	12
(2) Inoculated with sterile agar. Moist chamber	12	0
(3) Inoculated below ground with phycomycete	8	8
(4) Uninoculated	8	0
(5) Roots dipped in *Fusarium* sp. slurry	16	0
(6) Roots dipped in sterile water	14	0

The lesion produced commenced at the point of inoculation and spread both upwards and downwards. Twenty-four hours after symptoms first appeared the plants collapsed at ground level (Fig. 4). The phycomycete was reisolated from the lesion, and this isolate reproduced the disease. The phycomycete is therefore considered to be the cause of stem rot.

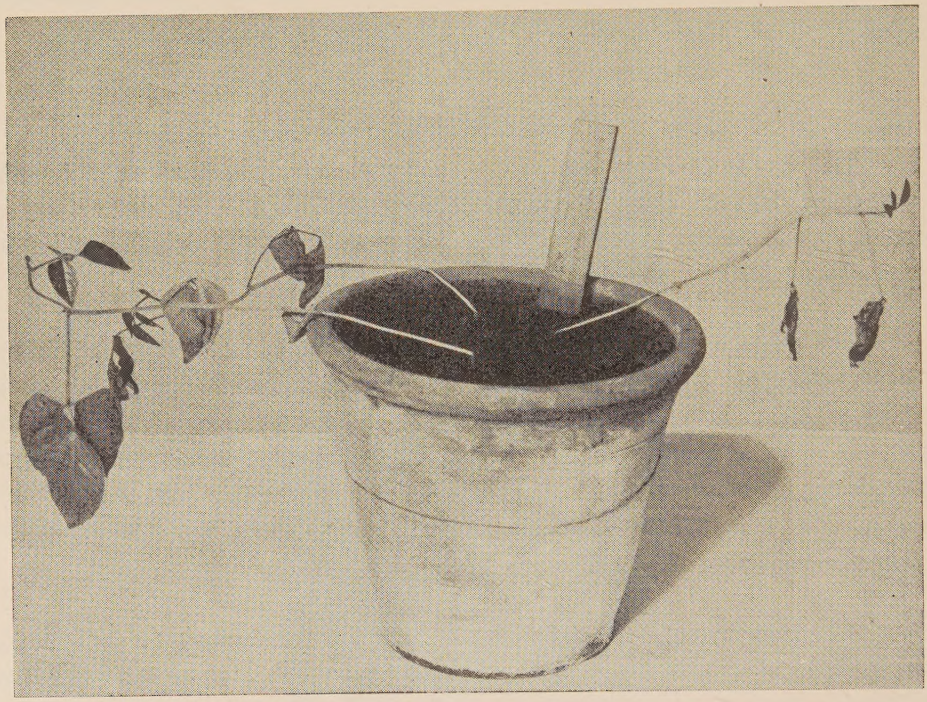

Fig. 4.

Cowpea Plants Inoculated Above Ground and Covered with a Bell-jar.

An alternative method of inoculation was tried with a view to establishing a technique which could be applied to large numbers of seedlings. This technique had been used previously by Weimer (1949). Inoculum growing on P.D.A. was cut into small cubes ($\frac{1}{4}$ in. square), which were placed against the uninjured stem just below the ground level and covered with soil. The pots were kept constantly moist. It was found that this technique yielded results equal to those obtained by inoculating above ground level and incubating in a moist chamber (Table 1 and Fig. 5). In addition, it was noted that the fungus produced a lesion which completely encircled the stem below ground level. Red stem canker, on the other hand, forms a narrow band which widens when it moves to the aboveground portion of the stem (Weimer 1949).

Other methods of inoculation were tried. In one, use was made of a culture of the fungus growing on a mixture of wheat and oats for soil inoculation. The mixture was added at the rate of 1 pint to a seedbox of sterilized soil measuring 18 in. x 15 in. x 5 in. Seed of the variety Poona was immediately sown in this soil. The fungus in this case attacked seedlings, producing a "damping-off" effect which killed all plants during the "two-leaf" stage. When in later work a resistant variety Blackeye 5 was uncovered, this technique was again used in comparing the reaction of Blackeye 5 and Poona. Although Blackeye 5 proved highly resistant under all other conditions, it damped-off to the same extent as Poona when tested in this manner. The technique was discarded for any future testing work.

Another technique tried involved the use of the fungus growing in a nutrient solution, which was poured on to the soil in which seedlings were growing. This technique, however, gave rather inconsistent results.

Fig. 5.

Cowpea Plants Inoculated Below Ground Without a Moist Chamber (left), and Uninoculated Plants (right).

IV. BIOLOGY OF THE COWPEA ORGANISM.

(1) Morphology.

Hyphae are hyaline, with occasional septa developing when old. Diameter of hyphae is very variable, with an average of 4·5 μ and with variations from 3 to 9 μ within the same hypha. Branching is mostly irregular and rather frequent.

The fungus forms both terminal and intercalary chlamydospores sparsely in distilled water, oatmeal agar (Tucker 1931) and maizemeal agar (Tucker 1931), but abundantly in Petri solution (Tucker 1931). These are variable in shape and size but mainly spherical, with an average diameter of 17 μ and a range of 12 to 21 μ.

Hyphal swellings are formed abundantly in Petri solution and oatmeal agar and rarely on maizemeal agar. These are variable in shape and size, usually roughly spherical and mostly intercalary with a few terminal.

Sporangia are never formed on solid artificial media and are produced only rarely when mycelium is transferred from pea broth (Leonian 1934) to distilled water. However, when cowpea nutrient broth (200 g. young green cowpea plants boiled in water, filtered, made up to 1,000 c.c., sterilized) is substituted for pea broth, sporangia are produced abundantly in the distilled water after 48 hours. Production of sporangia is sparse in Petri solution, but fairly abundant in non-sterile soil extract solution (Mehrlich 1935). Sporangia are produced abundantly when diseased cowpea stems are immersed in water for 24 hours.

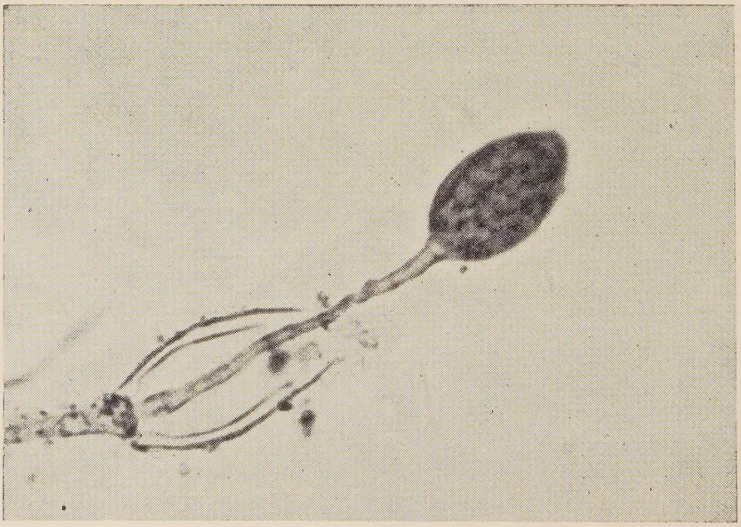

Fig. 6.

Phytophthora vignae. Nested and extended sporangium (X 367).

Sporangia growing in distilled water following growth in cowpea nutrient broth are borne mainly on simple undifferentiated sporangiophores, with a few on simple monochasial sympodia with proliferation. The latter type predominates on diseased tissue immersed in water, where an occasional compound sympodium also develops. Nested and nested and extended types have both been observed in monochasial sympodia (Fig. 6).

Table 2.

SIZE OF SPORANGIA OF THE COWPEA PHYCOMYCETE DEVELOPED ON VARIOUS SUBSTRATES.

Substrate.	Number Measured.	Range. (μ)	Average. (μ)	L/B ratio.
Diseased plants in field ..	50	24–69 x 15–39	49 x 27	1·8 : 1
Diseased plants immersed in water	100	30–72 x 18–54	58 x 32	1·8 : 1
Water following pea broth ..	50	24–60 x 15–30	37 x 22	1·7 : 1
Water following cowpea broth	50	30–57 x 15–27	42 x 21	2·0 : 1
Petri solution	20	30–61 x 21–36	40 x 30	1·3 : 1
	270	24–72 x 15–54	48 x 27	1·8 : 1

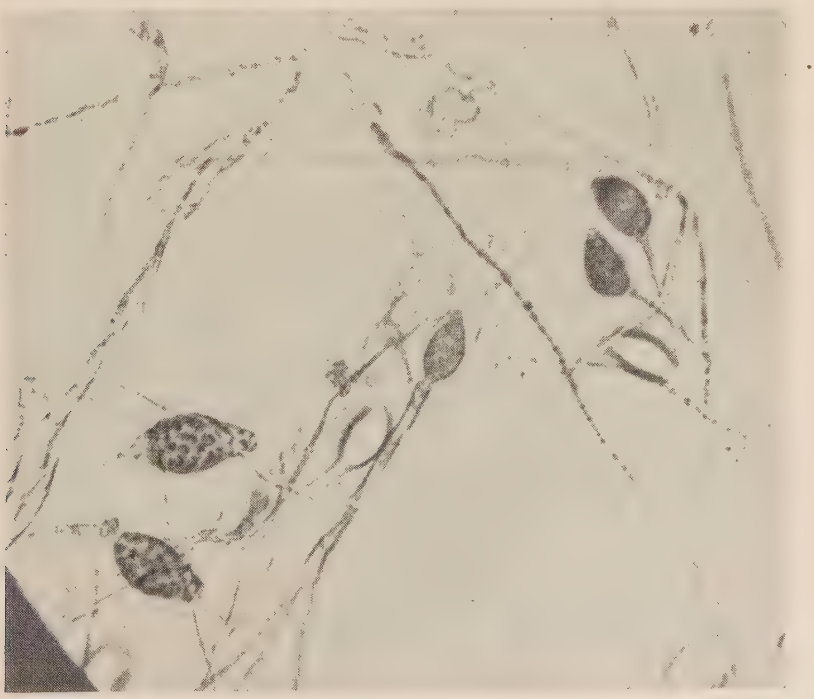

Fig. 7.

Phytophthora vignae. General View of Sporangia (X 170). Two sporangia showing zoospore differentiation.

Sporangia are very variable in shape and size but are mostly ovoid to ob-pyriform (Fig. 7). They are either non-papillate or very inconspicuously papillate. Measurements of sporangia developed on various substrates appear in Table 2.

Zoospores are differentiated within the sporangium (Fig. 7) and liberated singly through an apical pore. Up to 50 zoospores have been counted in a single sporangium. It is not uncommon to find a few zoospores trapped within the sporangial wall. On expulsion, the zoospores are reniform and very active. They become encysted after approximately half an hour. These encysted spores have an average diameter of 10 μ and germinate by a single germ tube after about two hours at room temperature.

Sexual organs are produced abundantly in host tissue (Fig. 8), oatmeal agar, maizemeal agar, and Petri solution and sterile water following growth in pea or cowpea broth. They are produced sparsely on malt extract agar (Leonian 1934), potato dextrose agar and cowpea extract agar (60 g. crushed cowpea seed, 17 g. agar, 1,000 c.c. water).

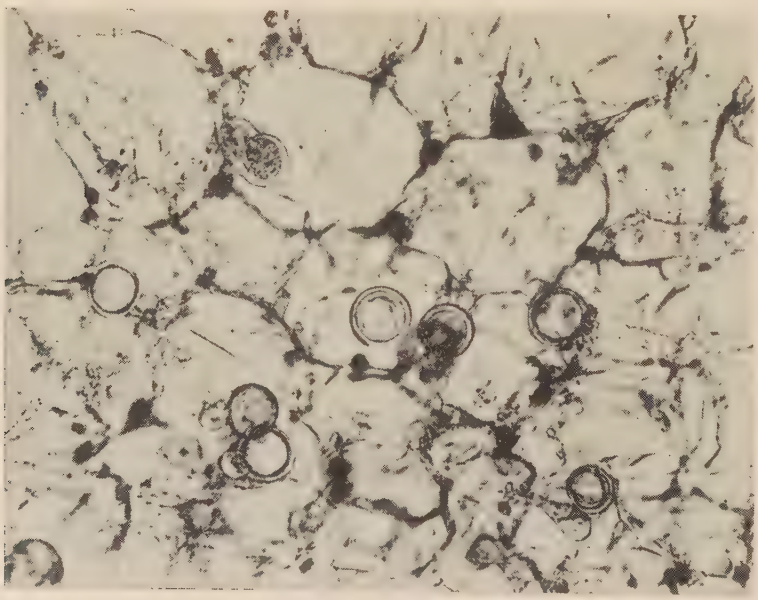

Fig. 8.

Sexual Bodies of *P. vignae* in the Host Tissue (X 180).

Antheridia are colourless, vary in shape from almost spherical to ovate, and measure 12-27 x 9-18 μ (av. of 250, 16 x 15 μ). They are almost exclusively amphigynous (Fig. 9).

Oogonia are colourless, smooth-walled and almost spherical in shape. They measure 27-42 x 24-46 μ (av. of 250, 33 x 31 μ). Oospores are colourless to light-brown, smooth-walled and spherical. They are slightly larger on artificial culture media than in host tissue, measuring 24-32 μ (av. of 175, 27 μ) on maizemeal agar and 21-30μ (av. of 148, 25μ) in diseased tissue.

The fungus is homothallic, both oogonia and antheridia being formed by hyphal tip cultures.

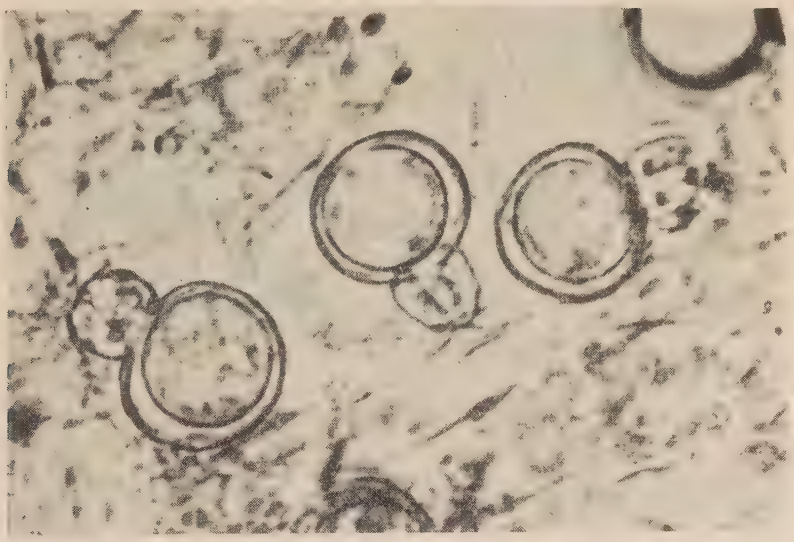

Fig. 9.

Phytophthora vignae. Oogonia, antheridia and oospores (X 533).

The oospore has a distinct outer wall, which is thin and much darker than the thick inner wall, the total width being approximately 3 μ. The oospores do not completely fill the oogonial cavity, in most cases not nearly so (Fig. 9).

(2) Histology.

The fungus is located chiefly in the parenchyma of the cortex, where it eventually causes a complete breakdown of the tissue, giving the typical sunken appearance to the diseased stem. A similar breakdown occurs in the pith. While the vascular tissue may also be invaded, there is no actual breakdown of the tissue. This may partly explain why even badly affected plants can remain green for long periods under favourable environmental conditions.

The mycelium is chiefly intercellular. Haustoria have been observed on rare occasions passing into cell tissue. Two types have been distinguished. The more common type is finger-like, being 3-5 μ long and 1·5-2·0 μ wide. The other type encountered is typically stud-shaped and measures up to 6 μ long

(Fig. 10). The haustoria are considered to be distinct from intra-cellular mycelium which is encountered frequently in young tissue and can also be seen passing through the thickest walls of the vascular vessels (Fig. 11). Where haustoria have been observed, the protoplasm of the cell has been either completely disintegrated or plasmolysed into a ball in a corner of the cell. Oospores are abundant in the diseased tissue (Fig. 8).

Fig. 10.

Stud-shaped Haustorium of *P. vignae* Passing into Host Cell (X 700).

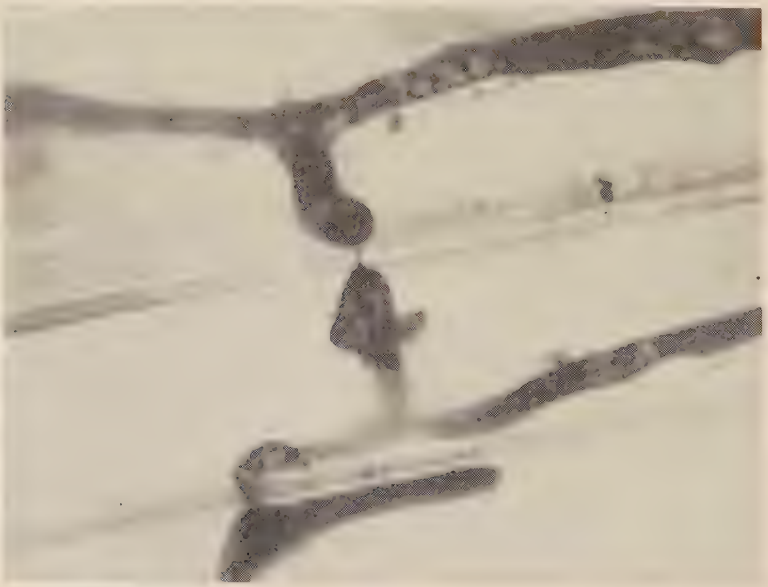

Fig. 11.

Mycelium of *P. vignae* Passing Through Thickened Walls of the Vascular Tissue (X 1260).

(3) Cultural Characteristics.

The fungus produces dense white colonies on potato dextrose agar at 27·5 deg. C., with moderate development of a low velvety mat of aerial mycelium and a definite margin. On malt extract agar, oatmeal agar and cowpea agar there is little development of aerial mycelium, and colonies are not dense. On maizemeal agar growth is rapid, with loose aerial hyphae.

Large, firm, brown lesions are produced on apple fruit (var. Granny Smith) following wound inoculations with this fungus. It is only slightly pathogenic to potato tubers.

The fungus makes good growth in a concentration of 1 in 4,000,000 malachite green but fails to grow in higher concentrations.

(4) Growth-Temperature Relationships.

The temperature relationships of this fungus were determined on maizemeal agar in a multi-temperature incubator giving a range of temperature from 0 deg. C. to approximately 40 deg. C. Uniform discs of inoculum ($\frac{1}{4}$ in. diameter) from a 5-day-old culture were centrally placed on the media in

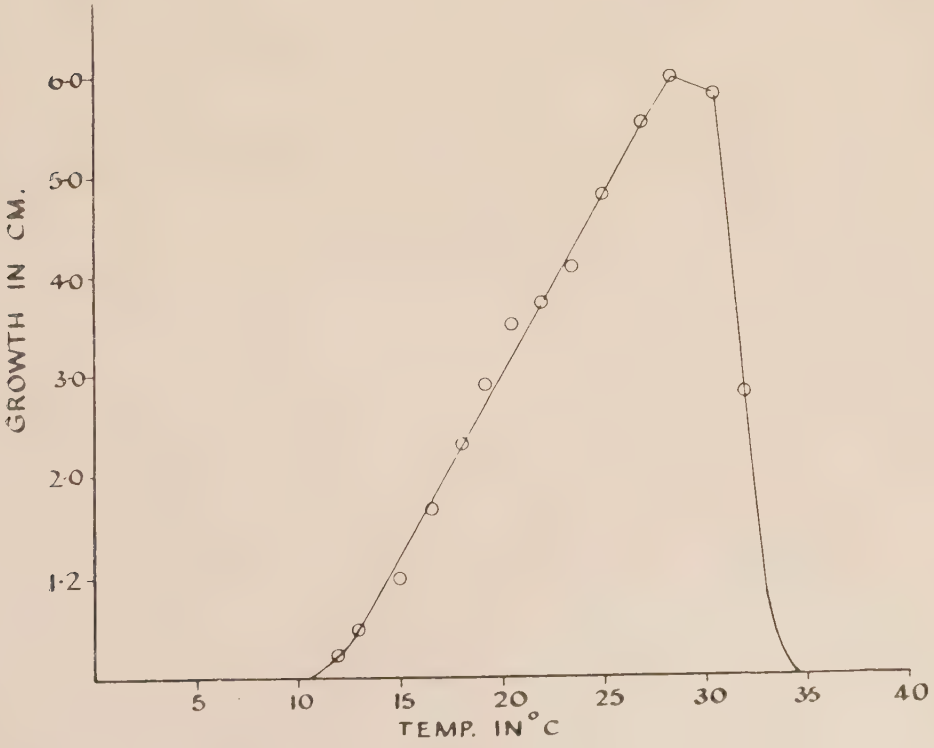

Fig. 12.

Growth-temperature Curve for *P. vignae.* 72 hours' growth on maizemeal agar.

4 oz. clear bottles and incubated for 24 hours at 27·5 deg. C. Measurement of the growth made in each bottle was recorded and three bottles were then placed in each of the 20 chambers of the multi-temperature range incubator. Growth of the colonies was measured at intervals of 24 hours. In Fig. 12 the growth made in 72 hours is expressed in graphical form.

This fungus has a minimum between 10 deg. and 12 deg. C., has an optimum from 28 deg. to 30 deg. C., and fails to grow at 34·5 deg. C. A culture of *P. cryptogea* Pethyb. & Laff. isolated from lucerne root rot and tested for comparison grew at a temperature below 5 deg. C., had an optimum of 24 deg. C., failed to grow at 34·5 deg. C., and grew only slightly at 32 deg. C.

V. IDENTITY OF THE COWPEA PHYCOMYCETE.

(1) Comparison with Other Species of Phytophthora.

General morphological characters and the nature of zoospore formation within the sporangium place this fungus in the genus *Phytophthora*.

Based on the key prepared by Tucker (1931) the fungus fits into the group of which *P. cryptogea* is the only member. It grows at 20 deg. C. on malt extract agar; has amphigynous antheridia; fails to grow at 35 deg. C. after four days on maizemeal agar; has sporangia mainly non-papillate, absent on ordinary agar media, but developing in Petri's mineral solution; has oospores that measure less than 30 μ and develop freely on solid media; and is pathogenic to wounded apple fruit.

It is, however, impossible to classify this organism as *P. cryptogea,* mainly because of the wide difference found in temperature responses. *P. cryptogea,* according to Tucker, grows well at 5 deg. C. and fails to grow at 32·5 deg. C. Results with the local isolate of the organism from lucerne agree with Tucker's observations. On the other hand, the cowpea organism makes no growth below 10·5 deg. C. and grows fairly well at 32·5 deg. C.

The cowpea isolate produces sexual organs very readily in three days on maizemeal agar and in one week on oatmeal agar. This is in direct contrast with the experience of Pethybridge and Lafferty (1919) and Tucker with *P. cryptogea,* the latter finding sexual organs only after exposure to winter temperatures at Columbia, Missouri. However, it is interesting to record that *P. cryptogea* is reported to exhibit the renewed growth of sporangiophores similar to that found in the cowpea organism and for this reason the two species are considered to be related. Tucker gave descriptions of another 19 species of *Phytophthora,* but this fungus fails to fit into any of the groups suggested by this author.

Leonian (1934) also prepared a key used for identification. In this key the organism falls into the group of which *P. boehmeriae* Saw. is the only member. The fungus makes good growth on malt extract agar at 20 deg. C., grows at 27 deg. C., has amphigynous antheridia, possesses oospores measuring less than 31 μ, and grows in the presence of 1 in 8,000,000 malachite green.

However, for two reasons it is impossible to accept this organism as *P. boehmeriae.* Firstly, *P. boehmeriae* produces sporangia readily on agar media (Tucker 1931; Freezi 1941) and the sporangia are distinctly papillate, having papilla 9-12 μ x 9-17 μ (Sawada 1927). Secondly, *P. boehmeriae* has antheridia which are nearly spherical, measuring 12-16 μ x 11-15 μ. The antheridia of the cowpea fungus are usually much larger than this. Dr. G. M. Waterhouse, who examined a culture, considered the size and shape of the antheridia to be a fairly constant feature and quite different from that of *P. boehmeriae.*

Attempts made to infect the leaves of *Boehmeriae nivea* Gaudich with this organism, using both P.D.A. culture cubes and a sporangial suspension in a moist chamber, were unsuccessful.

The close similarity in symptoms of red stem canker and stem rot necessitates a careful comparison of the causal organisms. The Queensland *Phytophthora* differs from *P. cactorum* as described by Weimer from cowpea in the following characteristics:—

——	*P. cactorum.*	Queensland *Phytophthora.*
Antheridia	Largely paragynous	Amphigynous
Oogonia	Av. 25·3 x 26·7 μ	Av. 33 x 31 μ
Oospores	Av. 20·8 μ dia.	Av. 26 μ dia.
Sporangia	Distinctly papillate	Non-papillate or very inconspicuously papillate

While the oogonial and oospore measurements of the two cowpea organisms differ considerably, they both fall within the range of variability found for *P. cactorum* by Tucker. The size difference, therefore, is not sufficient to exclude the Queensland fungus from this species. This leaves the differences in antheridia and sporangia, both of which are important from the point of view of classification. The difference in type of antheridia is of special importance, as both Tucker and Leonian regarded the presence of predominantly paragynous antheridia as a constant distinguishing feature of *P. cactorum.* As the Queensland *Phytophthora* always produces amphigynous antheridia, it is impossible to classify it as this species. Dr. G. M. Waterhouse has expressed the opinion that it is quite different from *P. cactorum.*

It is interesting to record here the similarity of temperature responses of *P. cactorum* isolated by Weimer from red stem canker and the local species of *Phytophthora.*

The organism has also been compared with descriptions of the following 17 species of *Phytophthora* described since 1931, but appears distinct from all of these.

Phytophthora primulae Tomlinson (Tomlinson 1952), *P. verrucosa* Alcock and Foister (Foister 1940), *P. macrospora* (Sacc.) Ito and Tanaka (Tanaka 1940), *P. quininea* Crandall (Crandall 1947), *P. inflata* Caroselli and Tucker (Caroselli and Tucker 1949), *P. porri* Foister (Foister 1931), *P. fagopyri* Takimoto nom. seminud (Ito and Tokunaga 1935) and *P. megasperma* Drechsler (Drechsler 1931) have either predominantly paragynous antheridia or at least a fair percentage of such antheridia. *P. quininea* also has large oospores (63 μ) and *P. megasperma* has larger oospores (av. 41·4 μ). In *P. himalayensis* Dastur (Dastur 1948) zoospores are unknown, sporangia are distinct and oospores are larger. *P. lateralis* Tucker and Milbrath (Tucker and Milbrath 1942) has large chlamydospores, sexual stages are absent, and growth is inhibited at 30 deg. C. *P. stellata* Shanor (Shanor 1938) has characteristic spiny oogonial walls. *P. formosana* Saw. (Sawada 1942a) has antheridia much smaller than those of this fungus. *P. leersiae* Saw. (Sawada 1941) has extremely large oogonia. *P. cinchonae* Saw. (Sawada 1936) and *P. murrayae* Saw. (Sawada 1942b) apparently have not been observed to form sexual spores and have papillate sporangia. *P. ricini* Saw. (Sawada 1942c) has smaller antheridia and papillate sporangia. *P. fragariae* Hickman (Hickman 1940) fails to grow at 30 deg. C.

(2) Description.

Because of the evidence presented, the fungus is considered to be a new species, for which the name *Phytophthora vignae* is accordingly proposed. English and Latin descriptions follow:

Phytophthora vignae n. sp.

Mycelium hyaline, non-septate when young, occasional septa developing when old; within host tissue mycelium mostly intercellular but some intracellular. Chlamydospores variable, mainly spherical, 12–21 μ (av. 17 μ), terminal and intercalary. Hyphal swellings in both solid and liquid media, irregular, both terminal and intercalary, single. Sporangia on host and in liquid nutrient media developed frequently on simple unbranched sporangiophores, frequently on a simple monochasial sympodial sporangiophore, rarely on a compound sympodial sporangiophore; sporangia variable in shape, mostly ovoid to ob-pyriform, 24–72 μ x 15–54 μ (av. 48 x 27 μ), non-papillate or inconspicuously papillate, germinating by zoospores; zoospores differentiated within sporangium, liberated singly, reniform when motile, spherical when encysted, average diameter of encysted zoospores 10 μ. Homothallic; sexual organs abundant in artificial media and host tissue; oogonia colourless, smooth, almost spherical, 27–42 x 24–46 μ (av. 33 x 31 μ). Antheridia amphigynous, variable in shape from almost spherical to ovate, 12–27 μ x 9–18 μ (av. 16 x 15 μ). Oospores colourless to light brown, 18–32 μ (av. 26 μ) in diameter, oogonial cavity not filled.

Habitat: In stem and root tissue of *Vigna sinensis* (L.) Endl. ex Hassk. in Queensland, causing a stem rot.

Phytophthora vignae n. sp.

Chlamydosporae variabiles, pro more sphaericae 12–21 μ (media 17 μ) terminales atque intercalares. Vesiculae hypharum in cultura atque solida atque liquida irregulares, terminales atque intercalares, solitariae. Sporangia in hospite et in cultura liquida in sporangiophoris vel simplicibus eramosisque vel monochasialibus sympodialibus proliferentibus, raro in sporangiophoro composito sympodiali enata; sporangia variabilia pro more ovoidea vel obpyriformia 27–72 μ x 15–54 μ (media 51 x 29 μ), epapillata vel subtilissime papillata; zoosporae singulae liberatae. Homothallica; genitalia plurima et in cultura artificiosa et in hospitae; oogonia sine colore, laevia, fere sphaerica 27–43 μ x 24–36 μ (media 33 x 31 μ). Antheridia amphigyna, e forma subsphaerica in ovatam variabilia 12–27 μ x 9–18 μ (media 16 x 15 μ). Oospora 18–32 μ (media 26 μ) diam. cavum oogonii haud impleta.

Habitat: In Queenslandia, intra caules radicesque *Vigna sinensis* (L.) Endl. ex Hassk.

VI. FACTORS AFFECTING DISSEMINATION AND EPIDEMIOLOGY OF THE DISEASE.

(1) Soil-borne Infection.

Numerous field observations have indicated that the disease is soil-borne. The most striking of these was on the Darling Downs during the summer of 1951-52. Half of a paddock of approximately 10 acres had been planted to cowpeas of the Poona variety during the 1950-51 season. In the 1951-52 season the farmer planted the whole paddock to the same variety. Stem rot caused a 50 per cent. loss on the land previously planted to Poona pea but only isolated loss on the other half of the paddock. In nearly every case of severe stem rot incidence the paddock has had a history of previous cowpea crops.

To test these observations experimentally, a simple pot experiment was designed in which soil collected from a badly diseased field was used. A drum of this soil was kept for 12 months in a glasshouse in which no cowpea plants had been grown. The soil was watered at fairly regular intervals and the surface soil only was allowed to dry out. At the end of the period half the soil was sterilized and placed in four sterilized pots. Another four sterilized pots were filled with the unsterilized soil. All were then sown at the rate of 10 seeds per pot with Poona cowpea seed harvested from healthy plants in a paddock free from the disease. Observations were taken; the results after 28 days appear in Table 3. All plants in the unsterilized soil showed typical symptoms of the disease within three weeks of germination (Fig. 13). All plants in the sterilized soil remained healthy.

Table 3.

RESULTS OF GROWING COWPEAS (VAR. POONA) IN SOIL FROM AN INFECTED FIELD AFTER 12 MONTHS' STORAGE.

Treatment.	Number of Plants Emerged per Pot.	Number of Plants per Pot with Stem Rot.
Sterilized	8	0
Unsterilized	8	8

Fig. 13.

Disease Development in Infected Soil Retained for One Year. Soil on the right sterilized.

This shows conclusively that the fungus can survive in moist soil for at least one season. Furthermore, field observations indicate that severe disease incidence can be expected where even two seasons have elapsed between the previous occurrence of the disease and the planting of another cowpea crop.

That the disease is soil-borne and does in fact build up in the soil as a result of growing successive crops of susceptible varieties has been brought out very strikingly by varietal trials conducted on infected soil. A plot of land at Boonah in south-eastern Queensland has been used for disease testing of cowpeas for a number of years. Table 4 indicates the incidence of disease in two varieties, Poona and Cristaudo, over a period of four years in replicated

and randomised trials during the 1952-53, 1953-54 and 1955-56 seasons. No trial was conducted during 1954-55, but the paddocks were sown to Poona cowpea with almost complete loss of plants.

Table 4.

Disease Incidence during Successive Seasons at Boonah.

Variety.	Percentage Infection Stem Rot.		
	1952–53.	1953–54.	1955–56.
Poona	60	90	100
Cristaudo	0	36	100

The figures indicate that there is a build-up of inoculum in the soil with successive plantings of susceptible varieties and that the variety Cristaudo requires a high level of inoculum in the soil to be severely attacked. This has been further demonstrated in other trials during the 1955-56 season and will be discussed in a later paper when field varietal trials are considered.

(2) Air-borne Infection.

Reference has already been made to the sporangia which frequently occur under moist conditions on disease lesions. It has been observed that the disease moves rapidly during periods of moist weather suited to the production of sporangia. It is considered that sporangia play a role in this spread, as there is a high incidence of aerial lesions during this type of weather. A simple trial was designed to test the ability of sporangia to reproduce the disease.

Diseased specimens of Poona and Reeves varieties were collected from a disease resistance trial in the Kingaroy district on Feb. 8, 1956. Clumps of sporangia on undifferentiated sporangiophores were seen emerging in great numbers from stomata on disease lesions. These were observed towards the active end of the lesion, in a zone extending downwards for a distance of up to two inches.

Two Poona cowpea plants growing in a pot sown on Jan. 6, 1956, were used for inoculation. One inch of the stem approximately half-way up each plant was marked off, using pieces of thin white thread tied loosely. This area, together with leaves attached to it, was surface sterilized by gentle application of 70 per cent. alcohol. Drops of sterile water were then placed on these areas and with the aid of a dissecting microscope sporangia were carefully picked from the diseased material and placed in the drops on one of the plants. Inoculations were carried out at 5 p.m. on Feb. 8. A bell-jar was placed over each pot. A sample of a drop on the leaf tissue was examined 16 hours later and large numbers of zoospores were seen. Some were still in

an active condition while others had germinated. A further examination of the surface of the old diseased lesions revealed many sporangia germinating directly, indicating that under conditions of high humidity the sporangia could act as spores.

The inoculated plant was examined at daily intervals. On Feb. 10 the belljar was removed. A slight water-soaking was noticed on the inoculated stem; by Feb. 13 this had developed into a typical aerial stem rot lesion (Fig. 14). *P. vignae* was isolated from the original diseased material and the inoculated stem tissue. No evidence of disease development was found on the inoculated leaf tissue and there was no lesion development on the neighbouring plant which had been sprayed with sterile water.

Sporangia are therefore capable of directly infecting stem tissue and this fact could account for the rapid spread of the disease under moist conditions. The disease has frequently been observed moving in a "front" across a paddock during moist weather (Fig. 15). It is probable that sporangia play a role in such spread.

(3) Effect of Water.

Examination of many infected fields has revealed that where plants are growing in drainage channels or areas of bad surface drainage the disease is usually much more prevalent than in the rest of the field. Detailed

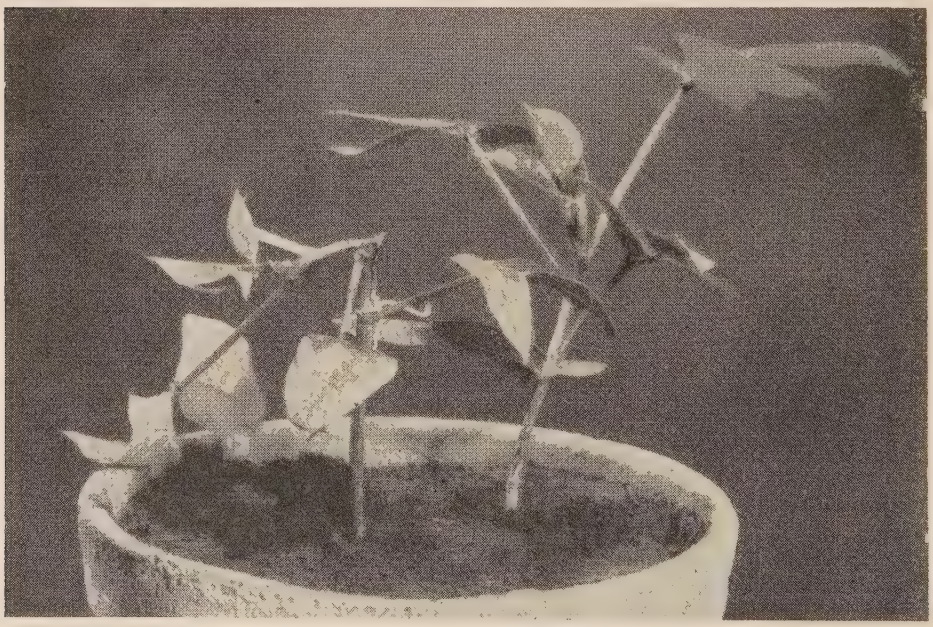

Fig. 14.

Sporangial Infection. Plant on left artificially inoculated with sporangia of *P. vignae* to produce aerial lesion.

Fig. 15.

Stem Rot Moving Along a Front Across a Paddock.

observations were recorded in a field of cowpeas on the South Coast of Queensland in which stem rot was spreading. The area was lowlying and had been artificially drained by means of furrows about a foot deep around the field. Twenty cowpea plants were scattered intermittently in these furrows, apparently as a result of the seed falling there during sowing operations. All of these plants were diseased, although stem rot was severe in only one corner of the paddock. It seems highly probable that the drainage furrow was acting as a medium for dissemination of the disease.

Another area close to Brisbane was traversed by shallow surface drains and the cowpea crop sown across the drains. Observations made in this paddock early in 1955 indicated that the drainage channels were aiding the dissemination of the disease. The plants in the channels were affected much earlier than the remaining plants and the general infection appeared to be spreading from these channels.

Numerous instances of the disease spreading from paddock to paddock down natural drainage channels have been observed.

During the 1955-56 season an opportunity to study disease incidence under varying conditions of free surface moisture was afforded in a varietal trial conducted on lightly infected soil in the South Burnett. The area had been sown to Poona cowpea during September 1955 and when examined some 10 weeks later was found to be infected with stem rot to the extent of approximately 5 per cent. of the plants. This infection was scattered lightly throughout the area.

The farmer ploughed this crop in almost immediately and a trial to assess the potentialities of 12 varieties was sown early in January 1956. Five replicated blocks were sown, each plot being a single half-chain row in which sufficient seed was sown to give approximately 100 plants. The block of land used was situated on an alluvial flat having a downward slope away from the creek bank. There was no fall at all in Block 1, a 12 in. fall in Block 2 and a 12 in. fall over the remaining three blocks. Conditions over each separate block were comparatively uniform. Heavy rains with light flooding of some of the trial occurred at intervals during the course of the experiment. Blocks 4 and 5 were swampy or lightly flooded for almost all the time after the first three weeks; Blocks 2 and 3 were swampy for short periods; but there was no excess water for any length of time on Block 1. Although full details of varietal reactions will be given in a later paper, the figures showing percentage infection of a susceptible variety (Poona) and a variety possessing some field resistance (Cristaudo) appear in Table 5. These figures have as far as possible been based on plants actually observed to have typical stem rot symptoms. However, some plants were in such an advanced diseased condition when examined that infection with *Phytophthora vignae* had to be assumed.

Table 5.

Percentage Infection of Two Varieties of Cowpeas on Infected Soil in the South Burnett.

Variety.	Percentage Infection.					
	Block 1.	Block 2.	Block 3.	Block 4.	Block 5.	Mean.
Poona	87	100	100	100	100	97
Cristaudo	17	57	64	100	100	68

In the planting the occurrence of waterlogging apparently played a leading part in the incidence of stem rot in the somewhat resistant variety Cristaudo. With the susceptible variety Poona, actual waterlogging of the soil was not necessary for severe attack.

While a high moisture level in the soil undoubtedly favours the spread of disease in soil previously not infested or only slightly infested, the position with soil carrying a heavy infestation is somewhat different. Under such conditions it is possible to get heavy losses from the disease under relatively

dry conditions. Such outbreaks have been observed on infertile hillside country in the Gympie and South Burnett areas. In order to measure this effect, an experiment was designed in which heavily infested soil was held at three different moisture levels and disease incidence recorded.

Soil was collected from an area that had been used as a disease testing garden about two months previously. This soil was thoroughly mixed and 5 lb. of the dry soil (calculated) was placed in each of 44 sterilized 7 in. pots. Eight of these pots of soil were then steam sterilized. Fourteen seeds of the variety Poona were sown in each pot and the pots placed on a bench in the glasshouse.

The soil was of a sandy nature and it was calculated that the addition of 16 fl. oz. of water would bring the soil to field capacity. Twelve of the pots of unsterilized soil were so treated (Group A). Twelve more had 12 oz. water added (Group B) and to 12 others 8 oz. was added (Group C). In addition, 3 pots of the sterilized soil were included in Group A, 3 in Group B and 2 in Group C for checking purpose.

Moisture levels were maintained by weighing every day and adding the required amount of water. It was noted in Group C that although germination was good subsequent growth of the plants was poor and the plants were often wilting prior to watering each day. In addition to stem rot caused by *Phytophthora vignae*, some losses were caused by a seedling foot rot (*Rhizoctonia* sp.) The trial was terminated 28 days after sowing. Relative incidence of the two diseases appears in Table 6.

Table 6.

Effect of Different Moisture Levels on the Incidence of Two Diseases of Cowpea in Naturally Infected Soil.

Moisture Level.	Soil Treatment.	Number of Plants.	Disease Infection after 28 Days.	
			Percentage *Rhizoctonia*.	Percentage *Phytophthora* Stem Rot.
Field capacity	Unsterilized ..	140	6	57
	Sterilized	36	0	0
¾ Field capacity	Unsterilized ..	142	15	67
	Sterilized	35	0	0
½ Field capacity	Unsterilized ..	153	33	58
	Sterilized	26	0	0

The results show that while decreasing the moisture level increased the incidence of seedling foot rot caused by *Rhizoctonia* sp., there was little effect on the incidence of *Phytophthora* stem rot. This experiment further confirms the field observations recorded above that moisture level does not have a great bearing on disease incidence when the inoculum level in the soil is high.

(4) Seed-borne Infection.

While it is probable that spread by air and water accounts for the movement of the disease within districts, it is difficult to explain the simultaneous occurrence of the disease in relatively isolated areas on the basis of these disseminating agencies. In addition, outbreaks of the disease have been reported on virgin land and land which has never previously grown cowpeas. The possibility of seed transmission has therefore been considered. Although detailed observations have been carried out over the last three seasons, no trace of any pod infection has been found. All attempts to infect pods artificially on the plant have to date been unsuccessful. Moreover, it is uncommon for a severely affected plant, with the lesion well advanced up the stem, to set seed. Consequently the collection of any quantity of seed from such plants has been impossible and this has limited the scope of any investigation.

Attempts have been made to determine whether the disease is seed-borne in the true sense of the term and whether it is transmitted in the trash with cowpea seed.

Initial testing involved plating seed from diseased plants onto malt extract agar. Seed was selected for all experiments from plants which had a lesion up to the tip of the stem. Eighty seeds were selected at random from each of eight seed sources and these were further divided into lots of 20 seeds. Four different treatments were then given to these lots of seed:—

(1) Wetted in 95 per cent. alcohol and immersed in a 1 per cent. solution of sodium hypochlorite for 10 minutes followed by two washes of sterile water.

(2) Wetted in 95 per cent. alcohol, immersed in 0·1 per cent. mercuric chloride for five minutes and washed twice in sterile water.

(3) Washed twice in sterile water.

(4) Untreated.

The seed was then plated out onto malt extract agar, half the seed in each treatment being cut open prior to plating.

In every treatment a number of fungi were isolated. These were all of the quick-growing type, with species of *Alternaria* predominating. Other fungi isolated were *Macrophomina phaseoli* fairly consistently, *Phomopsis* sp., *Penicillium* sp., *Fusarium* sp. and a number unidentified. In no case was *Phytophthora* sp. isolated. However, the test cannot be considered conclusive because it is highly probable that any colony of *Phytophthora* would be quickly overgrown by one of the quicker-growing organisms and its identity lost.

The second series of experiments consisted of planting unsterilized seed from infected plants in sterilized soil. Seed lots from four different sources each totalling 100 seeds were planted in a separate box of sterilized soil in a

glasshouse away from any possible source of contamination. Of the 383 plants germinating only two became diseased, the disease being due to *Rhizoctonia* infection in the seedling stage.

The third experiment involved incorporating trash from diseased cowpea plants in sterilized soil prior to planting seed of a susceptible variety. Dead diseased plants stored for up to three months have been used effectively to inoculate soil for varietal testing purposes. However, for the experiment being described the trash used was obtained from the pods and pod stalks only of the infected plants from which seed was collected for the previous two experiments. This is the normal type of trash occasionally encountered with cowpea seed, which is, in the main, remarkably free from such contamination. Seed of the variety Poona was sown in the soil so contaminated and of the 110 plants which grew two developed a foot rot in the seedling stage. *Macrophomina phaseoli* was isolated from these diseased plants. No further loss of plants occurred. In view of the frequent occurrence of *M. phaseoli* on maturing pods and stalks it is not surprising that there are losses from this fungus.

In these experiments no evidence has been found to show that the disease is transmitted by seed. The possibility still remains, but the difficulty of obtaining any quantity of seed from infected plants handicaps further investigations. Further testing is, however, contemplated.

In order to determine the effect of seed treatment on the incidence of disease in infested soil, a small trial was conducted in the glasshouse. Soil was collected from a field of cowpeas badly diseased with stem rot and this was placed in earthenware pots. Four seed treatments were used, these being 50 per cent. thiram dust, Tetroc (chloronil 98 per cent.), Agrosan (1·0 per cent. Hg) and untreated. All dusts were applied at the rate of 2 oz. per bushel. Three pots were sown with each treatment, 10 seeds being used in each pot. In addition, three of the pots of soil were steam sterilized and sown with untreated seed. The pots were maintained at an even moisture level, and disease incidence was recorded at regular intervals. The results appear in Table 7.

Table 7.

Effect of Seed Treatment on Incidence of Stem Rot in Infested Soil.

Treatment.	Total Germination.	Total Infection.	
		After 15 Days.	After 30 Days.
Thiram dust (50% thiram)	28	18	28
Tetroc (98% chloronil)	29	21	29
Agrosan (1·0% Hg)	30	20	30
Untreated	26	23	26
Soil steam sterilized	29	0	0

Seed treatment apparently has no effect in reducing incidence of stem rot in infested soil.

Should evidence be obtained to support seed-borne infection, this subject of seed treatment may have to be further considered.

VII. TEMPERATURE AND DISEASE DEVELOPMENT.

During the course of varietal testing in the glasshouse it was noted that it was difficult to obtain infection during the cooler months of the year. In order to investigate this matter further an experiment was carried out to trace the percentage infection of cowpea plants during the autumn, winter and spring months.

Naturally infested soil was collected from the disease nursery and thoroughly mixed before placing in earthenware pots. A number of these pots were then sterilized to act as controls. Sowings commenced on Mar. 8, 1955, and were repeated at approximately fortnightly intervals until June 6. A further sowing was made on Sept. 16, 1955. On each occasion, eight unsterilized pots and three sterilized pots were sown with 10 seeds of the variety Poona. All pots before and after sowing were maintained by daily watering at a moisture level of 22·5 per cent., this being close to the field capacity of the somewhat sandy soil used.

During the course of the experiment daily recordings were made of the maximum and minimum soil temperature by means of thermometers inserted into sterilized soil through holes drilled half-way down the side of the pot. The

Table 8.

INCIDENCE OF STEM ROT (*P. vignae*) ON COWPEAS IN RELATION TO TIME OF SOWING.

Date of Sowing.	Percentage Infection After											
	1 Week.	2 Weeks.	3 Weeks.	4 Weeks.	5 Weeks.	6 Weeks.	7 Weeks.	8 Weeks.	10 Weeks.	12 Weeks.	14 Weeks.	16 Weeks.
8–3–55	5	34	70	80	88	90						
22–3–55	9	36	42	42	73	83						
4–4–55	7	13	41	..	51	55	..	61	65*			
18–4–55	4	12	22	24	26	35	39	44	56	57*		
11–5–55	7	11	16	20	25	33	34	34	39	41	41	41*
23–5–55	0	19	20	22	25	26	29	32	32	32	32*	
6–6–55	0	4	9	9	9	11	..	..	..	..	30*	
16–9–55	0	3	10	25	43	60	83					

*Surviving plants had reached maturity.

mean temperature for each week was calculated from these recordings. Similar recordings were made of air temperatures, but it was found that the variation between air temperature and soil temperature was very slight. The mean soil temperature has therefore been used in all comparisons.

Weekly recordings were made of the number of healthy and diseased plants in each treatment (Table 8) and of the growth of plants in the control pots. The results have been expressed graphically in Fig. 16.

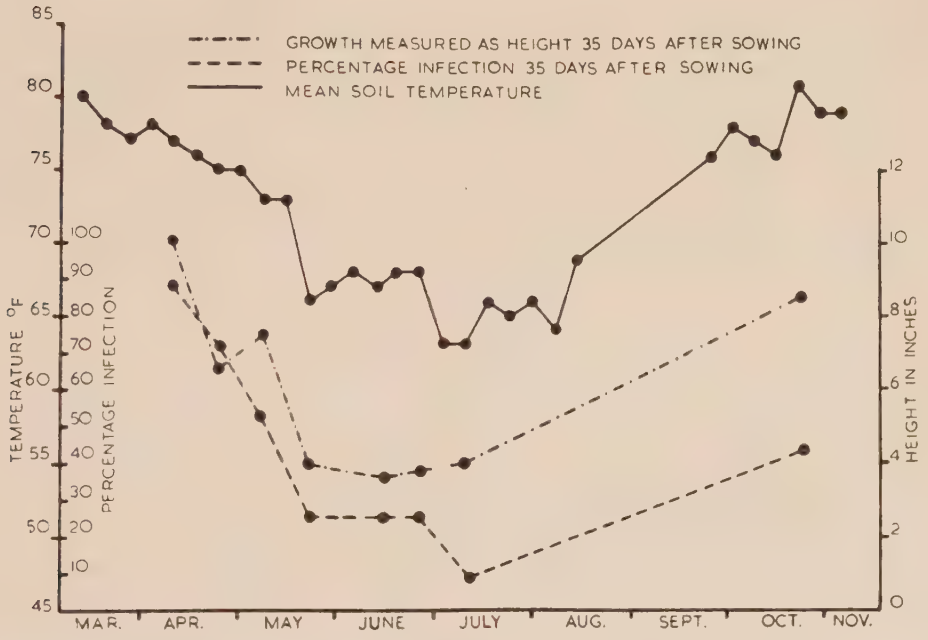

Fig. 16.

Relationship of Soil Temperature and Time of Sowing to the Incidence of Stem Rot and Rate of Growth of Cowpeas.

Stem rot caused by *P. vignae* proved the major cause of plant loss. An occasional plant died as a result of infection with *Rhizoctonia* sp., but such plants were not considered when determining the percentage infection. For the purpose of comparison a graph has been drawn to express the percentage loss 35 days after sowing in relation to the curve for the mean soil temperature. The height of plants after 35 days is also shown.

It will be seen that date of sowing has had a marked influence on both percentage infection and growth rate. That the effect has not been entirely one of delayed infection owing to slower growth of the host is demonstrated by the relatively low percentage infection at maturity for the sowings of Apr. 4 and 18, May 11 and 23 and June 6 (Table 8). Thus in these particular

sowings a greater percentage of plants survived and completed their life cycle than in the earlier sowings. The fall in infection closely follows the curve of mean soil temperature and it appears highly probable that there is a correlation between these two variables. The optimum temperature for growth of the fungus lies between 28 deg. C. and 30 deg. C. (82 deg. F. and 86 deg. F.) (Fig. 12). Although the temperature limits for growth of the fungus are comparatively wide—11–34 deg. C. (52–93 deg. F.)—it appears that the limits of its ability to function as an aggressive parasite are somewhat narrower than these.

The rise in percentage infection with the advent of warmer conditions in September and October indicates that the fungus has "overwintered" quite satisfactorily in the soil. Although there appears to be a slight initial lag in the disease incidence of the September planting, the rise in percentage infection is later particularly rapid (Table 8).

To attempt to test this effect of temperature on infection under more controlled conditions, the multi-temperature incubator used previously for determining the growth rate of *P. vignae* was employed. The 20 chambers gave a range of temperature from 0 deg. to 40 deg. C.

Nutrient agar was placed in the bottom inch of 6 in. x 1 in. flat-bottomed glass tubes and these were sterilized after plugging with cotton-wool. Two cowpea seeds of the variety Poona were then sown in each tube. The tubes were placed in sunlight until germination was complete and the plants had fully developed their seedling leaves. Five days after sowing the resulting seedlings were inoculated with *P. vignae* by placing a $\frac{1}{4}$ in. square of a P.D.A. culture on the stems just above the level of nutrient agar. Two tubes were then placed in each of the chambers of the multi-temperature incubator. Observations on lesion development were made.

Lesions appeared on the plants at 28 deg. C. on Sept. 16, and by Sept. 17 both plants had collapsed. The plants at 19·0, 21·5, 23·0, 24·5 and 26·0 deg. C. became infected and had collapsed by Sept. 21. No further lesion development was recorded. All plants above 34·0 deg. C. died, apparently as a result of high temperature conditions. The experiment was continued until Sept. 30 and by this time the remaining seedlings at all temperatures above 10 deg. C. were badly etiolated, while those below this temperature had little growth. Those results again serve to emphasise the rather narrow limits of temperatures in which aggressive parasitism of *P. vignae* can be expected—in this experiment 19–28 deg. C. (66–82 deg. F.)—compared with the range of temperature over which the fungus can grow—11–34 deg. C. (52–93 deg. F.).

VIII. ACKNOWLEDGEMENTS.

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ROOT-KNOT NEMATODE CONTROL INVESTIGATIONS IN TOBACCO IN QUEENSLAND

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SUMMARY.

During 1948-55, 8 field trials were set out in North Queensland to investigate the control of the root-knot nematode Meloidogyne javanica **(Treub) Chitwood, which is a serious pest of tobacco.**

It is shown that, irrespective of the use of nematocides in the field, even a trace of nematode galling in seedlings can be primarily responsible for appreciable economic losses.

In heavily infested irrigated fields, yields and appraised values can be increased more than threefold by the correct use of either DD or EDB.

Where tobacco is grown each year on the same site, galling tends to increase at a greater rate than where resistant crops are used in the rotation. This use of resistant crops, however, does not prevent consistently an increase in galling: one of the reasons may be the impracticability of obtaining pure stands of resistant plants.

I. INTRODUCTION.

The recognised problems with root-knot nematode as a pest of tobacco in Queensland have been the complete protection of seedlings and the protection of field plants at least during establishment and the early growth stages. Most of the earlier literature on tobacco pests, including nematodes, is consolidated in articles by J. H. Smith (1931, 1933), Veitch (1938, 1942), Officers of the Department of Agriculture and Stock (1951) and W. A. Smith (1952).

Lamb (1892) and subsequent authors recommended firing for nematode control in seedbeds and the method has been the commercial practice for over half a century. Haney (1950), following American thought at that time, tested chemical methods for use in seedbeds. The results indicated that when the three essentials of weed control, plant growth and nematode control were considered, firing was still the best treatment. In view of the continued general success of firing, no further major effort was made to screen chemicals in seedbeds. For future guidance, however, a trial was carried through to the field to demonstrate the effect of seedling infestation on yields.

As effective nematocides were not available until recent years, recommendations for nematode control in the field were necessarily restricted to maintaining plant vigour by cultivation and fertilizing, uprooting crop residues after harvesting, rotating with immune crops, building up the soil organic matter, and eliminating chance pest distribution. All these practices may have helped in some degree with crop production in infested land, but none has been proved reliable.

The field investigations in North Queensland recorded in this paper were carried out during the period 1948–1955, principally at Claredale in the Lower Burdekin district. These consisted of seven trials with nematocides, the examination of root infestation in a crop rotation trial (see Appendix), and observations on commercial control.

During the 1950-51 season in the Bundaberg and Miriam Vale districts, C. D. Brickhill set out field trials to test DD levels and placement positions, and found that commercial nematode control was possible in rain-grown tobacco when treatment was restricted to the row position (unpublished reports, Queensland Department of Agriculture and Stock).

II. SPECIES INVOLVED.

Prior to 1954, root-knot nematode was referred to by Queensland authors first as *Heterodera radicicola*, then as *Heterodera marioni* (Cornu) Goodey. Smith (1954), anticipating Colbran (1955), used the name *Meloidogyne* spp. Recent root-knot nematode surveys by Colbran (unpublished reports, Queensland Department of Agriculture and Stock) show that only *Meloidogyne javanica* (Treub) Chitwood has been recorded from tobacco in northern Queensland, and this is the species dealt with in this paper.

III. MATERIALS.

The nematocides used were:—

DD.—A commercial mixture of 1,3 dichloropropene and 1,2 dichloropropene.

EDB.—Ethylene dibromide: used in power kerosene, 1 pint EDB made up to 1 gallon (EDB (Sp. G. $\frac{25}{25}$ 2·170) content 12·5 per cent. v/v).

CBP.—Chlorobromopropene.

IV. METHODS.

(1) General.

For the trial with infested seedlings a seedbed 20 ft. x 4 ft. was fired correctly at one end, grading off to an ineffective burn at the other. Seedlings were hosed out, and the roots floated in water for grading into the categories required. Only clean seedlings from fired beds were used in the other trials.

All the field trial plantings were watered by furrow irrigation. Precautions were taken during land preparation to prevent excessive movement into treated parts of soil which might contain nematodes and also within plots where nematocide placement was restricted. In the 1948-49 screening trial at Mareeba, DD with and without an emulsifier was applied in the irrigation

water and by injection. In subsequent trials only the injection method was used. A light commercial hand injector metering fumigant by piston strokes from a $\frac{1}{2}$-gal. tank provided reasonable accuracy in treating the field plots. Strings marked at intervals of 1 ft. were used as a guide during injection. The depth of injection was determined by a stop-plate fixed 6 in. above the spear outlet holes.

All experimental tobacco was grown in rows 4 ft. apart, and treatments were made 2-3 weeks before planting out. Three types of placement were used —the "4 line", requiring injection lines 6 in. and 18 in. each side of the row position; the "2 line", 6 in. each side; and the "1 line", on the row position.

The dosage rates used in successive trials varied until 20 gal. per acre was selected as a standard. The rates per acre actually used with the equivalent rates per foot are shown in Table 1.

Table 1.

NEMATOCIDE DOSAGE RATE EQUIVALENTS.

gal./ac.	ml./ft.		
	4 line.	2 line.	1 line.
33·3	3·5	..	..
20·0	2·1	..	..
19·1	2·0	..	..
16·7	1·8	..	..
10·0	..	2·1	4·2
9·6	1·0	..	..
8·1	..	1·7	..

No water-sealing of injected plots was practised except in the 1948-49 screening trial, when a watering can was used for the purpose.

Soil temperatures during treatment in all trials were mild and no adverse effects due to this factor were expected or noticed. Control of insect pests, according to current Departmental recommendations, was implemented as required in all trials.

Layout, plot size, details of the various treatments and other relevant information are given with the results for each trial.

(2) Assessing Results.

(a) Yields.

Yields from the 1950-51 Claredale trials were taken as weight of harvested green leaf per plant. Stand counts were made and the leaf was picked as it ripened.

It was found that relative green weights may vary more than the corresponding cured leaf weights, so yields from the later trials are expressed in economic units. As the crop ripened, plots were harvested separately and labelled sticks of leaf were cured and afterwards bulked in parcels. When ready for grading, the parcels were weighed and recorded by plots. The sum of grade weights is given in the 1953-54 trial results as the yield of cured leaf after grading. This differs from the yield before grading by variation in moisture content and loss of trash.

Samples of each grade were given an assessed value by a trade appraiser, and relevant plot values were calculated accordingly. The appraised value is the only criterion which takes into account the effects of nematode infestation on quality as well as on quantity of tobacco leaf.

(b) Root-Knot Indices.

The method used to assess the degree of root galling, usually after harvesting, is essentially that of Smith and Taylor (1947). The root-knot index for a plot, calculated after rating the roots of each plant, is an estimate of percentage infestation.

V. RESULTS.

(1) Field Trial with Infested Seedlings.

The categories used to distinguish nematode infestations of seedlings were :—

Clean—free from galls.

Trace—with one or two minute galls.

Distinct—with one or two distinct galls.

Moderate—with distinct galling distributed over several roots.

Table 2.

YIELDS AND APPRAISED VALUES.

(Field trial with infested seedlings.)

Soil Treatment.	Seedling Infestation.	Yield of Cured Leaf Before Grading (lb./ac.).	Appraised Value (£/ac.).
	Clean	992	449
	Trace	702	274
	Distinct	632	304
	*Moderate	..	..
DD 20 gal./ac. 4 line	Clean	1,700	976
DD 20 gal./ac. 4 line	Trace	1,652	870
DD 20 gal./ac. 4 line	Distinct	1,260	631
DD 20 gal./ac. 4 line	Moderate	578	286
Necessary differences for significance 5%	..	277	107
Necessary differences for significance 1%	..	374	144

* This treatment was not included in the analyses because of shortage of seedlings of the correct rating.

This trial was planted out on Oct. 5, 1954, as a (4 x 2) x 5 randomised block with a plot size of 2 rows x 47 ft., adjacent to the 1954-55 nematocide trial at Claredale. Yields and appraised values are given in Table 2. Root-knot indices were not obtained, as fungal breakdown following heavy rains soon after harvesting caused severe root destruction.

Differences in plant growth were not apparent until the approach of harvesting. At that time the degree of wilting present was inversely related to the yields obtained.

(2) 1948-49 Screening Trial, Mareeba.

The light-grey sandy soil had grown tobacco two years previously. Plot size was 4 rows x 32 ft. in a 5 x 5 randomised block layout. Planting was carried out on Oct. 23–25. Root-knot indices only, taken from 20 plants in the two inner rows of each plot, were obtained; they are given in Table 3.

Table 3.

ROOT-KNOT INDICES.

(1948–49 Screening Trial, Mareeba.)

Treatment.	Mean Root-Knot Index.
DD injected 33·3 gal./ac. 4 line	7·2
DD injected 16·7 gal./ac. 4 line	25·6
DD with emulsifier in irrigation water 16·7 gal./ac. ..	38·8
DD in irrigation water 16·7 gal./ac.	47·0
Control	30·4
Necessary difference for significance 5%	18·5
Necessary difference for significance 1%	25·5

The DD when run into the irrigation water settled on the bottom of the furrows and probably evaporated when the water soaked in.

(3) 1950-51, Claredale.

Three sites were used on the light-textured soils of the Burdekin association. Plot size was 2 rows x 36 ft., and each trial was a 4 x 6 randomised block. Two of these were to test row treatments simultaneously with Brickhill's work in southern Queensland, and the third to check the application of DD at the lower strength which had failed at Mareeba the previous season.

The trials, planted during late September and early October, were subjected to unseasonable heavy rains which caused some waterlogging and reduced plot yields. The results are given in Tables 4 and 5.

Table 4.

YIELDS.

(1950–51, Claredale.)

Treatment.	Green Leaf Yields (lb./plant).		
	Trial I.	Trial II.	Trial III.
DD 19·1 gal./ac. 4 line	1·46	1·14	1·26
DD 8·1 gal./ac. 2 line	1·31	1·12	..
DD 9·6 gal./ac. 4 line	..	..	1·21
Control	1·27	0·88	0·94

Differences are not significant.

Table 5.

ROOT-KNOT INDICES.

(1950–51, Claredale.)

Treatment.		Mean Root-Knot Indices.		
		Trial I.	Trial II.	Trial III.
DD 19·1 gal./ac. 4 line		26·0	22·2	9·4
DD 8·1 gal./ac. 2 line		66·9	59·4	..
DD 9·6 gal./ac. 4 line		..	..	29·5
Control		84·2	86·8	85·1
Necessary differences for significance	5%	25·2	17·8	11·6
	1%	35·8	25·2	16·4

The efficacy of DD in preventing root galling was not reflected in the yields, probably due to the weather factor. Overall yields were low.

(4) 1953-54, Claredale.

A 6 x 5 randomised block layout was used, with a plot size of 2 rows x 70 ft. The soil type was similar to that for Trial I, 1950-51, the two blocks being adjacent.

Planting out took place during late September, and early difficulties in establishment led to some unevenness in plant stand. The results are given in Table 6.

Untreated plants were not as tall as the others but the difference was not outstanding and was not apparent before flowering. Mild wilting and premature ripening of leaf were noticeable in the control plots from about the time of the first main flowering.

Table 6.

YIELDS, APPRAISED VALUES AND ROOT-KNOT INDICES.

(1953–54, Claredale.)

Treatment.	Mean Yield Cured Leaf After Grading (lb./ac.).	Appraised Values (£/ac.).	Mean Root-Knot Index.
EDB 20 gal./ac. 4 line	1,482	722	37·5
EDB 10 gal./ac. 2 line	1,569	747	46·1
DD 10 gal./ac. 2 line	1,460	662	58·3
DD 20 gal./ac. 4 line	1,619	789	63·0
DD 10 gal./ac. 1 line	1,538	707	71·5
Control	826	313	96·8
Necessary differences for significance 5%	264	119	16·7
Necessary differences for significance 1%	359	162	22·8

The tolerance of the tobacco plant under irrigated conditions to moderate root-knotting as indicated by the indices is reflected in these results. The value of the nematocides, and of the 2 line and 1 line placements, is obvious when nematode infestation is severe.

(5) 1954-55, Claredale.

This trial was planted in September on Burdekin fine sand which had carried a heavily infested crop during the first half of the year. A 6 x 4 randomised block layout with a plot size of 2 rows x 84 ft. was used, with control strips of 2 rows x 80 ft. through the block in place of randomised control plots. Results are given in Table 7.

Table 7.

YIELDS, APPRAISED VALUES AND ROOT-KNOT INDICES.

(1954–55, Claredale.)

Treatment.	Mean Yield Cured Leaf Before Grading (lb./ac.).	Appraised Value (£/ac.).	Mean Root-Knot Index.
EDB 20 gal./ac. 4 line	1,979	811	19·4
EDB 10 gal./ac. 2 line	1,639	718	22·9
EDB 10 gal./ac. 1 line	1,610	673	24·7
DD 20 gal./ac. 4 line	1,552	666	25·6
DD 10 gal./ac. 2 line	1,499	651	29·6
DD 10 gal./ac. 1 line	1,105	469	59·4
Control	(675)	(196)	(74·0)
Necessary differences for significance 5%	418	207	16·2
Necessary differences for significance 1%	580	286	22·2

No early stunting was observed in the untreated plants. During harvesting the leaves ripened rapidly and prematurely, and frequently wilted before picking.

(6) Screening of CBP.

During the 1954-55 season at Claredale a 4 x 5 randomised layout with control strips was used to screen CBP against DD as the standard. Plot size was a single row of 10 plants.

Plants were pulled for root-knot assessment six weeks after planting out. The indices given in Table 8 are therefore not comparable with those figures from the other trials.

Table 8.

Root-Knot Indices.

(Screening of CBP.)

Treatment.	Mean Root-Knot Index.
DD 20 gal./ac. 4 line	0·0
CBP 10 gal./ac. 4 line	2·6
CBP 15 gal./ac. 4 line	1·1
CBP 20 gal./ac. 4 line	6·9
Control	(13·3)
Necessary difference for significance 5%	3·2

The nature of the variation indicates an inadequacy in the small-plot technique. CBP, however, does not give promise of improving on DD, and is a pungent liquid most unpleasant to handle.

VI. DISCUSSION.

Results from the field trial with infested seedlings clearly demonstrate (Table 1) that only clean seedlings should be planted out. Irrespective of the use of nematocides in the field, even a trace of nematode galling in tobacco seedlings can be primarily responsible for appreciable economic losses.

A high percentage of Queensland tobacco land is infested in some degree by root-knot nematodes, and there has been a tendency to throw the worst of this land out of tobacco production. No ready method of sampling the pest populations at the time of planting, to help predict nematode damage in the subsequent crop, was available to the author. In commercial practice, district and field histories are used for this purpose. All trial sites had recent histories of heavy infestations and the results show that under these circumstances the use of nematocides in irrigated crops can increase yields, as appraised values, more than threefold (Table 7). Concurrent with this work, soil fumigating

machines were evolved, mostly by local effort, for translating experimental results into the commercial sphere. W. A. Smith (1954) first firmly recommended the field use of nematocides, and again the next year as follows:—

> ". . . fumigate the soil when free from weeds and in good tilth with EDB or DD at least three weeks before planting out. The recommended dosages per acre are 10 gal. of 12½% EDB or 10 gal. of DD. Fumigation depth should be 6 inches, with thorough sealing after application.
>
> Where careful soil working can be achieved during the early life of the crop, the intended planting line may be treated by a single application along the centre of the row, or one 6 inches each side of it. The double line treatment makes allowance for the lateral movement of soil in and near the planting lines during working. Treatments in rows 1 foot apart over the whole field, requiring 20 gal. of fumigant per acre, permit maximum lateral working of soil, but if reasonable care is taken this more expensive treatment is unnecessary."
>
> . . . . .
>
> "Soil fumigants and detailed dosage rates are:—
>
> (1) *EDB (12½% concentration).*
> *Machine Application.*
>
> Treatment in the centre of row position—1 pint to 2 1/16 chains (136 feet),
>
> *or,*
>
> Treatment 6 inches each side of row position—1 pint from each outlet to 4⅛ chains (272 feet). The same rate applies if 20 gallons are used in rows 1 foot apart across the field.
>
> *Hand Injector Application.*
>
> Same positions as for machine application but with injections 1 foot apart. Rate: 1 fluid ounce per each 7 injections for centre of row treatment, and 14 injections totalling 1 fluid ounce for the other treatments.
>
> (2) *DD.*
>
> Use the commercial material at the same rates as for 12½% EDB."

At present most tobacco in Queensland is irrigated, and the conclusion from general field observations is that increased yields, comparable with experimental results, are obtainable when strict attention is given to control details as recommended above.

VII. ACKNOWLEDGEMENTS.

Facilities for conducting the trials were provided by the Agriculture Branch of the Department of Agriculture and Stock, and by several farmers. Mr. G. W. Saunders (Assistant Entomologist) was in charge of the 1954-55 trials and other Departmental officers assisted with all trials. The statistical analyses were carried out by Mr. P. B. McGovern (Biometrician, Division of Plant Industry).

All this assistance is gratefully acknowledged.

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APPENDIX.

Root-Knot Nematode in a Crop Rotation Trial.

A crop rotation trial on the Clare Tobacco Experiment Farm, Claredale, provided an opportunity to record the extent of root galling on tobacco in six successive years. The land was open forest on the Burdekin levee, cleared and prepared in the first half of 1948. It had previously been used as grazing land. The trial was arranged as an 8 x 4 randomised block with a plot size of 8 rows x 56 ft.

Appendix Table 1.

ROOT-KNOT INDICES.

(Crop Rotation Trial.)

Rotation.	Year.					
	1948.	1949.	1950.	1951.	1952.	1953.
1	5·0	12·6	64·0	50·7	85·0	79·3
2	5·4	9·7	66·7	42·6	75·1	49·6
3	2·8	9·6	32·8	49·0	75·8	88·5
4	13·7	..	..	67·6	..	..
5	5·5	..	29·1	..	..	27·7
6	6·1	22·2	..	..	62·5	..
7	14·0	..	26·2	..	49·6	..
8	10·2	38·4	..	43·6	..	66·3

The year was divided into three equal cropping periods. Before each tobacco crop the land was bare-fallowed for one period. Other crops in the annual series of rotations were volunteer weeds (Table 1, Rotation 1), Gambia pea (*Crotalaria goreensis* Guill. & Perr.) (Rotation 2), and peanuts (*Arachis hypogaea* L.) (Rotation 3). A cycle of Gambia pea, potato (*Solanum tuberosum* L.) and 20 months of Rhodes grass (*Chloris gayana Kunth*) preceding tobacco was repeated in three time phases (Rotations 4, 5, 6). Twelve months of Gambia pea and 4 months of maize (*Zea mays* L.) was repeated in two time phases (Rotations 7, 8).

In plant species composition surveys towards the end of April in 1950, 1952 and 1953 it was found that all plots carried a variety of weeds either as a trace or in larger numbers. The Rhodes grass plots carried fewer weeds than most others.

Each tobacco crop was pulled after harvesting, and root-knot indices were assessed. The results are given in Appendix Table 1.

Despite wide variation in behaviour between plots, a general increase in root-knot galling with tobacco cropping was demonstrated. None of these variations could be readily explained from general observations, but broad conclusions may be drawn from the data in Table 1. Where tobacco is grown each year on the same site, galling tends to increase at a greater rate than where resistant crops are used in a rotation. This use of resistant crops, however, does not prevent consistently an increase in galling: one of the reasons may be the impracticability of obtaining pure stands of resistant plants.

APPLE COOL STORAGE INVESTIGATIONS IN 1955

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Division of Plant Industry.

SUMMARY.

Investigations have confirmed the importance of maturity with regard to the storage life of Granny Smith apples both in normal air storage and in controlled atmosphere storage.

As in earlier experiments, the incidence of storage disorders was closely related to the age of the tree from which the fruit was picked.

Superficial scald incidence was again closely related to the amount of carbon dioxide present in the storage atmosphere, but under conditions of high carbon dioxide concentrations the effect of maturity on the incidence of this disorder was most pronounced.

Wastage in fruit picked at the correct stage of maturity was reduced by controlled atmosphere storage and the fruit kept firmer.

Delaying the storage of fruit reduced scald incidence but increased wastage from mould and soggy breakdown and resulted in softer fruit.

The most satisfactory storage atmosphere was a mixture of 16 per cent. oxygen and 5 per cent. carbon dioxide. In 1954, when earlier picking dates were used, a mixture of 10 per cent. oxygen and 2.5 per cent. carbon dioxide was found to be the most satisfactory, as fruit in this atmosphere did not develop as much scald as in the higher concentration of carbon dioxide. This was the case again for early picks in 1955. However, fruit picked at the later picking date developed less scald and when stored under a 16 per cent. oxygen plus 5 per cent. carbon dioxide mixture was firmer than that stored under the 10 per cent. oxygen plus 2.5 per cent. carbon dioxide mixture.

Ozone-generating ultra-violet lamps did not have any effect on the incidence of storage disorders.

I. INTRODUCTION.

Within recent years it has become particularly important to the apple industry in Queensland for the crop to be marketed over a fairly long period. Accordingly, since 1951 problems of cool storage, particularly of the Granny Smith variety, have been investigated in Queensland.

Progress in these experiments has been reported briefly by Trout in the Annual Reports of the Queensland Department of Agriculture and Stock for the years 1951-52 to 1955-56, and more extended progress reports have appeared in "Queensland Fruit and Vegetable News," a weekly paper published by the Committee of Direction of Fruit Marketing.

The studies have been concerned with the effects of stage of maturity at picking and age of tree on the keeping quality of Granny Smith and Delicious varieties and with the effect of storage atmosphere on the Granny Smith.

The 1955 investigations reported here covered the following aspects:—

(1) Delicious

(*a*) Effects of maturity and tree age on keeping quality.

(2) Granny Smith

(*a*) Effects of maturity and tree age on keeping quality.

(*b*) Effects on keeping quality of ozone-generating ultra-violet lamps fitted in a store equipped with a system of air purification.

(*c*) Effects of controlled storage atmospheres on keeping quality.

(*d*) Effects on keeping quality of delays prior to storage in controlled atmospheres.

II. EXPERIMENTS WITH DELICIOUS.

(1) Experimental Details.

Fruit of three maturities was used, the picking dates being:—

First pick (Maturity 1) .. Feb. 14, 1955
Second pick (Maturity 2) .. Feb. 23, 1955
Third pick (Maturity 3) .. Mar. 7, 1955

The experimental fruit was obtained from six orchards in the Granite Belt surrounding Stanthorpe. Eighteen half-bushel cases were obtained from each orchard, comprising three half-bushel cases of each maturity from old and young trees. The fruit was wrapped in oiled wraps, packed, and stored in the C.O.D. Cool Store, Thulimbah, on the Granite Belt, at 34-36 deg. F. Removals of fruit from store were made on Aug. 1 (Removal 1), Sept. 5 (Removal 3) and Oct. 3 (Removal 3).

After removal from store, the cases were railed to Brisbane, held there at air temperature for seven days to simulate normal marketing conditions, and then inspected for storage disorders.

Firmness was measured by means of the Magness Penetrometer. Five fruits from each case were taken as a sample and readings were made on opposite sides of the fruit with the 7/16 in. plunger. The mean of the 10 readings was recorded.

(2) Results.

The results are presented in Table 1.

Only a small number of fruit was affected by *mould*. Fruit from Removal 1 had less mould than from Removal 3, and mould was more prevalent in fruit from young trees than in that from old trees. Degrees of maturity of the fruit did not affect its susceptibility to mould.

Table 1.

ADJUSTED MEAN PERCENTAGES OF DEFECTS, AND FIRMNESS, OF DELICIOUS APPLES AFTER REMOVAL FROM COOL STORE.

——	Mould.	Superficial Scald.	Total Wastage.	Firmness.
Removal 1—				
Maturity 1	0·5	16·3	16·8	13·04
Maturity 2	1·6	4·3	6·2	12·58
Maturity 3	0·6	Nil	0·6	11·46
Old trees	0·3	3·7	4·0	12·08
Young trees	1·7	6·7	8·6	12·64
Removal 2—				
Maturity 1	1·2	34·4	36·0	14·12
Maturity 2	1·0	5·9	6·9	12·62
Maturity 3	1·3	0·1	1·6	11·71
Old trees	1·0	7·2	8·4	12·72
Young trees	1·2	13·6	14·8	12·92
Removal 3—				
Maturity 1	1·9	34·5	36·5	15·21
Maturity 2	2·0	9·4	11·9	14·29
Maturity 3	2·0	0·1	2·6	12·96
Old trees	1·4	8·8	10·6	13·94
Young trees	2·6	15·6	18·5	14·36

Mould : Removal 1 sig. less than Removal 3 (1% level).
Old trees sig. less than Young trees (5% level).

Superficial Scald : Maturity 2 sig. less than Maturity 1 (1% level).
Old trees sig. less than Young trees (1% level).
Removal 1 sig. less than Removal 2 (5% level).
Removal 1 sig. less than Removal 3 (1% level).

Total Wastage : Maturity 3 sig. less than Maturity 2 and 1 (1% level).
Maturity 2 sig. less than Maturity 1 (1% level).
Removal 1 sig. less than Removal 3 (1% level).
Removal 1 sig. less than Removal 2 (5% level).
Old trees sig. less than Young trees (1% level).

Firmness : Removal 3 sig. greater than Removal 2 and Removal 1 (1% level).
Removal 2 sig. greater than Removal 1 (1% level).
Maturity 1 sig. greater than Maturity 2 and 3 (1% level).
Maturity 2 sig. greater than Maturity 3 (1% level).
Young trees sig. greater than Old trees (1% level).

In previous years no *superficial scald* developed in the variety Delicious, but in the 1955 investigations a considerable amount was present. Fruit from the third pick was not affected by scald, and that from the second pick showed less wastage than that of earlier harvested fruit. There was more scald in fruit from young trees and also an increase in the incidence of the disorder with increase in storage time.

Total wastage from mould and scald decreased with increase of maturity at picking and increased with storage time. Fruit from old trees had less wastage than that from young trees.

The *firmness* of the fruit on removal was affected by maturity, storage time and age of tree. Fruit remained firmer the earlier it was picked. On the other hand, the fruit increased in firmness with length of storage; this is at variance with results of previous tests. Fruit from young trees was firmer than that from old trees; this did not support results obtained previously.

The increase in firmness with increase in storage period and the fact that fruit from old trees was softer than that from young trees cannot be satisfactorily explained. However, it had been noticed on previous occasions that towards the end of the storage period of the variety Granny Smith some withering occurred, particularly with fruit from young trees. It is possible that moisture loss accompanying withering makes the fruit more resistant to penetration by the test plunger and this may account for the increasing firmness of the fruit.

(3) Discussion.

From the experiments carried out in 1953 and 1954, it appeared that for cool storage the optimum picking date for Delicious apples grown on the Granite Belt was between Feb. 25 and Mar. 3. The results of the 1955 experiments largely confirm this finding, but since scald did not develop in fruit from the third pick, it would appear that the later part of the period would be the safer. It was again demonstrated that, for long storage, fruit from old trees is much more suitable than that from young trees because it remains firmer and is less susceptible to breakdown.

III. INVESTIGATIONS WITH GRANNY SMITH.

(1) Effects of Maturity and Tree Age on Keeping Quality.

(a) Experimental Details.

This experiment was a continuation of investigations commenced in 1953. The three picking dates were, however, somewhat later than those used in previous years, because it had been shown that there was a considerable reduction in the incidence of superficial scald with increasing maturity.

The three picking dates in 1954 were:—

First pick (Maturity 1)	Mar. 16–18
Second pick (Maturity 2)	Mar. 30–Apr. 2
Third pick (Maturity 3)	Apr. 12–14

The 1955 picking dates were:—

First pick (Maturity 2)	Mar. 28
Second pick (Maturity 3)	Apr. 12
Third pick (Maturity 4)	Apr. 27

It will be noted that the first pick in 1955 is designated Maturity 2, the second Maturity 3 and the third Maturity 4. This is because it is proposed to combine all the results in one report at the completion of the investigations and this nomenclature of maturities was designed to avoid confusion.

Eighteen half-bushel cases were obtained from each of six growers, and comprised three half-bushel cases at each of the picking dates from old and young trees. The fruit was wrapped in oiled wraps, packed, and stored at 34–36 deg. F. in the C.O.D. Cool Store, Thulimbah. Removals from store were made on Oct. 3 (Removal 1), Nov. 7 (Removal 2) and Nov. 28, 1955 (Removal 3).

After removal from the cool store, the fruit was railed to Brisbane, held at air temperatures for seven days to simulate normal marketing practices, and then inspected for storage disorders.

(b) Results.

The results are given in Table 2.

Table 2.

ADJUSTED MEAN PERCENTAGES OF DEFECTS, AND FIRMNESS, OF GRANNY SMITH APPLES AFTER REMOVAL FROM AIR COOL STORAGE.

—	Mould.	Soggy Breakdown.	Superficial Scald.	Total Wastage.	Firmness.
Removal 1—					
Maturity 2 ..	4·6	0·3	7·4	13·4	12·50
Maturity 3 ..	12·5	0·6	7·9	21·8	12·25
Maturity 4 ..	5·7	0·4	0·8	7·0	12·62
Old trees	3·7	0·3	6·5	11·8	12·42
Young trees ..	11·2	0·4	3·4	15·2	12·50
Removal 2—					
Maturity 2 ..	3·7	8·4	14·4	27·2	11·88
Maturity 3 ..	5·6	12·1	13·4	33·2	12·00
Maturity 4 ..	5·8	6·3	1·0	13·2	11·83
Old trees	3·4	8·4	8·5	21·8	11·94
Young trees ..	6·6	9·1	10·1	26·2	11·86
Removal 3—					
Maturity 2 ..	5·0	30·7	4·3	41·7	10·88
Maturity 3 ..	5·9	31·0	5·6	44·2	10·35
Maturity 4 ..	8·5	17·0	3·1	30·2	10·75
Old trees	4·5	18·8	7·2	33·2	10·46
Young trees ..	8·6	33·5	1·4	44·1	10·86

Mould : Old trees sig. less than Young trees (1% level).

Soggy Breakdown : Removal 2 sig. less than Removal 3 (1% level).
Old trees sig. less than Young trees (5% level).

Superficial Scald : Removal 3 sig. less than Removal 2 (5% level).
Maturity 4 sig. less than Maturity 2 and 3 (5% level).
Young trees sig. less than Old trees, all removals (5% level).
Young trees sig. less than Old trees, Removal 1 (5% level).
Young trees sig. less than Old trees, Removal 3 (1% level).
No sig. difference between Young trees and Old trees, Removal 2.

Total Wastage : Removal 1 sig. less than Removal 2 and 3 (1% level).
Removal 2 sig. less than Removal 3 (1% level).
Maturity 4 sig. less than Maturity 2 and 3 (1% level).

Firmness : Removal 1 sig. greater than Removal 2 and 3 (1% level).
Removal 2 sig. greater than Removal 3 (1% level).

Although only a small number of fruit was affected, fruit from young trees was more susceptible to *mould* wastage than fruit from old trees. Mould did not increase with length of storage, nor was the susceptibility of the fruit to mould affected by the maturity of the fruit.

There was a considerable increase in the incidence of soggy breakdown with storage time. The occurrence of breakdown was not affected by the time of picking, but fruit from old trees was less susceptible than that from young trees.

The effect of maturity on *superficial scald* was very marked, as the average wastage was approximately 0·5 per cent. for the third pick, compared with 5·6 per cent for earlier picks. The later picking dates in 1955 resulted in much less scald wastage than that in previous years. This effect of decrease in the incidence of superficial scald with increase in maturity has been reported by Padfield (1950), although it was claimed by Tindale and Huelin (1939) that the reduction in scald occurs only over a short period, after which there is a marked increase in the disorder with further increase in maturity. Previously, no variation in the susceptibility of fruit from trees of different ages was observed, but in 1955 fruit from young trees had less scald than that from old trees in two of the three removals.

Fruit from the third pick showed less *total wastage* than that from either of the other two picks, and increase in storage time increased the total wastage. No significant difference due to age of trees was recorded.

The only factor that affected *firmness* of the fruit was the length of storage, the fruit becoming softer with time of holding.

(c) Discussion.

In previous years, the most suitable picking period for storage of the variety Granny Smith was during the first two weeks of April. Fruit picked towards the end of this period developed less scald during storage, but this advantage was offset by an increase in the other storage disorders together with softening of the fruit. However, the 1955 results showed that picking the fruit later considerably reduced the incidence of superficial scald, and firmness was retained without any increase in wastage from mould or soggy breakdown. Further investigations will be necessary to determine whether these effects are seasonal or due to the later picking date.

(2) Effects on Keeping Quality of Ozone-generating Ultra-violet lamps.

In view of claims of beneficial results in cool stores equipped with ozone-generating utra-violet lamps, the effects of such equipment on the storage disorders of Granny Smith apples were investigated. The cool store in which the experiment was conducted is fitted with an air purification system employing activated charcoal as the volatile absorbent. Investigations by Trout (1951) with this variety showed that air purification alone did not give any less wastage

than the normal method of storage, so any beneficial storage results which have been claimed could be attributed to the ultra-violet lamps. A number of ultra-violet lamps were fitted throughout the experimental chamber.

(a) Experimental Details.

Three picking dates were used:—

First pick (Maturity 2)	Mar. 28
Second pick (Maturity 3)	Apr. 12
Third pick (Maturity 4)	Apr. 27

Table 3.

Adjusted Mean Percentages of Defects, and Firmness, of Granny Smith Apples after Removal from a Cool Store Equipped with Ozone-generating Lamps.

—	Mould.	Soggy Breakdown.	Superficial Scald.	Total Wastage.	Firmness.
Removal 1—					
Maturity 2 ..	2·4	0·4	10·1	12·9	13·31
Maturity 3 ..	3·5	Nil	2·6	7·1	13·19
Maturity 4 ..	8·9	Nil	Nil	9·7	13·38
Old trees	3·8	0·2	1·9	7·2	13·42
Young trees ..	6·0	Nil	6·6	12·6	13·17
Removal 2—					
Maturity 2 ..	3·9	6·8	15·5	27·6	11·81
Maturity 3 ..	7·2	13·9	5·0	26·7	11·56
Maturity 4 ..	9·7	6·2	4·9	21·2	12·38
Old trees	4·5	4·8	3·3	14·3	12·00
Young trees ..	9·9	13·6	14·3	37·8	11·83
Removal 3—					
Maturity 2 ..	3·1	37·4	12·8	53·4	11·06
Maturity 3 ..	7·1	34·7	3·7	46·4	11·38
Maturity 4 ..	9·6	20·6	0·7	31·2	11·62
Old trees	3·6	20·2	7·8	32·4	11·08
Young trees ..	10·0	41·8	3·1	55·1	11·62

Mould : Maturity 3 sig. less than Maturity 4 (5% level).
Maturity 2 sig. less than Maturity 4 (1% level).
Maturity 2 sig. less than Maturity 3 (5% level).
Old trees sig. less than Young trees (1% level).

Soggy Breakdown : Removal 2 sig. less than Removal 3 (1% level).
Old trees sig. less than Young trees (1% level).

Superficial Scald : Maturity 4 sig. less than Maturity 2 (1% level).
Maturity 3 sig. less than Maturity 2 (5% level).

Total Wastage : Removal 1 sig. less than Removal 2 and 3 (1% level).
Removal 2 sig. less than Removal 3 (1% level).
Old trees sig. less than Young trees (1% level).

Firmness : Removal 1 sig. greater than Removal 2 (1% level) and Removal 3 (5% level).
Removal 2 sig. greater than Removal 3 (5% level).

Eighteen half-bushel cases were obtained from each of four growers and comprised three half-bushel cases at each of the picking dates from old and young trees. The fruit was wrapped in oiled wraps, packed, and stored at 32–33 deg. F. in a store on the Granite Belt. Removals were made on Oct. 3 (Removal 1), Nov. 7 (Removal 2) and Nov. 28, 1955 (Removal 3).

After removal from the cool store, the fruit was railed to Brisbane, held at air temperature for seven days to simulate normal marketing delays, and then inspected for storage disorders.

(b) Results.

The results appear in Tables 3 and 4.

Table 4.

Adjusted Mean Percentages of Total Wastage of Granny Smith Apples after Removal from a Store Equipped with Ozone-generating Lamps (Store B) and from Normal Air Storage (Store A).

	Removal 1.		Removal 2.		Removal 3.	
	Store A.	Store B.	Store A.	Store B.	Store A.	Store B.
Maturity 2	13·4	12·9	27·2	27·6	41·7	53·4
Maturity 3	21·8	7·1	33·2	26·7	44·2	46·4
Maturity 4	7·0	9·7	13·2	21·2	30·2	31·2
Old trees	11·8	7·2	21·8	14·3	33·2	32·4
Young trees	15·2	12·6	26·2	37·8	44·1	55·1

Old trees sig. less than Young trees (1% level).
Removal 1 sig. less than Removal 2 (1% level).
Removal 2 sig. less than Removal 3 (1% level).
Maturity 4 sig. less than Maturity 3 (1% level).
Maturity 4 sig. less than Maturity 2 (5% level).

Mould infection increased with stage of maturity at picking but not with storage time. Fruit from old trees was less affected than that from young trees.

The incidence of *soggy breakdown* increased with length of storage but was not greatly affected by time of picking. Fruit from old trees was less affected than that from young trees.

Fruit from the second and third picks had less *superficial scald* than that from the first pick. Time of storage and age of tree had no effect.

Total wastage increased with length of storage. There was no effect of time of picking on total wastage, but fruit from old trees had less wastage than that from young trees.

The only factor which affected the *firmness* of the fruit was length of storage, the fruit becoming softer with time of holding.

(c) Discussion.

These results are in agreement with those obtained with fruit from the same sources and used for investigations into the effects of tree age and maturity on the storage behaviour of this variety. Table 4 shows a comparison of the total wastage from both stores obtained by adding the figures for mould, superficial scald and soggy breakdown.

There were no differences in wastage between fruit from the two stores. Fruit from Store B was ½ lb. firmer than fruit from Store A. The difference is not of commercial importance.

(3) Effect of Controlled Atmosphere Storage on Keeping Quality.

(a) Experimental Details.

In experiments conducted in 1952, 1953 and 1954 there was a very definite relationship between the incidence of superficial scald and the carbon dioxide concentration of the storage atmosphere, and also a considerable decrease in the incidence of scald in fruit from the later pickings. The 1955 investigations were designed to determine the effects of later picking dates on the incidence of superficial scald.

For this purpose 36 half-bushel cases were obtained from each of four growers on the Granite Belt, comprising three half-bushel cases from young and old trees at two picking dates. The fruit was stored in three different atmospheres—normal air storage, 10 per cent. oxygen plus 2·5 per cent. carbon dioxide, and 16 per cent. oxygen plus 5 per cent. carbon dioxide. These were the atmospheres used in 1954 and were retained because of the good results obtained. The two picks correspond with the second and third picks used in the other Granny Smith investigations and were:—

First pick (Maturity 3)	Apr. 12
Second pick (Maturity 4)	Apr. 27

After picking, the fruit was wrapped in oiled wraps, packed, and stored at 34–36 deg. F. in the C.O.D. Cool Store, Thulimbah, in airtight containers. The atmospheres within the storage cabinets were allowed to build up to the approximate concentrations, and then adjusted with nitrogen and carbon dioxide. During the storage period regular checks on the concentrations of oxygen and carbon dioxide in the cabinet were made by means of an Orsat gas analyser and any necessary adjustments made with nitrogen or carbon dioxide. Removals of fruit from the cabinets were made on Oct. 3 (Removal 1), Nov. 7 (Removal 2), and Nov. 28, 1955 (Removal 3). After each removal was made, the atmospheres in the cabinets were re-adjusted to the required concentrations with nitrogen and carbon dioxide.

The fruit, after removal from store, was railed to Brisbane, where it was held at air temperature for seven days to simulate normal marketing delays and then inspected for storage disorders.

(b) Results.

The results are set out in Table 5.

Table 5.

ADJUSTED MEAN PERCENTAGES OF DEFECTS, AND FIRMNESS, OF GRANNY SMITH APPLES AFTER REMOVAL FROM CONTROLLED ATMOSPHERE STORAGE.

—	Mould.	Soggy Breakdown.	Superficial Scald.	Total Wastage.	Firmness.
Control—					
Maturity 3 ..	10·2	13·8	6·3	32·0	11·76
Maturity 4 ..	6·5	4·5	0·5	12·3	11·60
Old trees	3·9	8·5	3·3	17·8	11·63
Young trees ..	12·7	9·1	2·9	25·0	11·73
Removal 1 ..	9·8	0·2	2·0	12·7	12·59
Removal 2 ..	6·4	7·9	5·8	21·3	11·88
Removal 3 ..	8·0	19·8	1·6	31·3	10·58
10% O_2 ; 2·5% CO_2—					
Maturity 3 ..	5·4	5·9	22·6	35·4	13·44
Maturity 4 ..	5·5	3·5	1·5	10·7	13·19
Old trees	3·4	3·7	12·0	20·6	13·29
Young trees ..	7·4	5·3	9·9	23·0	13·33
Removal 1 ..	3·9	0·3	7·3	12·4	14·28
Removal 2 ..	6·9	2·6	13·3	23·9	13·09
Removal 3 ..	5·5	11·6	12·9	30·6	12·56
16% O_2 ; 5% CO_2—					
Maturity 3 ..	5·5	2·7	31·4	40·4	13·58
Maturity 4 ..	5·1	1·4	2·1	10·7	13·48
Old trees	4·1	1·1	14·2	21·3	13·31
Young trees ..	6·4	3·0	16·3	26·7	13·75
Removal 1 ..	3·7	0·1	11·5	15·9	14·31
Removal 2 ..	4·9	1·1	16·2	22·8	13·41
Removal 3 ..	7·3	7·1	18·2	34·2	12·88

Mould : 16/5 sig. less than Control (5% level).
Old trees sig. less than Young trees (1% level).

Soggy Breakdown : 16/5 sig. less than Control (1% level).
10/2·5 sig. less than Control (5% level).
Removal 2 sig. less than Removal 3 (1% level).

Superficial Scald : Control sig. less than 10/2·5 and 16/5 for Maturity 3 (1% level).
No sig. diff. between atmospheres for Maturity 4.
Control sig. less than 10/2·5 and 16/5 for age of trees and storage time (1% level).

Total Wastage : Removal 1 sig. less than Removal 2 (5% level).
Removal 2 sig. less than Removal 3 (5% level).
Removal 1 sig. less than Removal 3 (1% level).
Maturity 4 sig. less than Maturity 3 (1% level).

Firmness : Removal 1 sig. greater than Removal 2 and 3 (1% level).
Removal 2 sig. greater than Removal 3 (1% level).
10/2·5 and 16/5 sig. greater than Control (1% level).

Mould wastage was greater in fruit from young trees than in fruit from old trees. Fruit stored in the 16 per cent. oxygen plus 5 per cent. carbon dioxide mixture had less wastage than the control fruit; the other storage atmosphere showed no difference from the control.

The incidence of *soggy breakdown* was low irrespective of maturity, tree age or composition of the atmosphere. No soggy breakdown was present in fruit from the first removals, but it increased with length of storage. The age of the trees had no bearing on the incidence of the disorder.

An increase in *superficial scald* with an increase in carbon dioxide concentration was observed in fruit not fully mature. This effect has been noted in all controlled atmosphere experiments carried out with this variety in Queensland since 1952. Huelin and Tindale (1947) observed the same effect with controlled atmosphere storage of Victorian Granny Smith apples. In the early work of Brookes, Cooley and Fisher (1919) it was reported that an accumulation of carbon dioxide was not responsible for the incidence of superficial scald; rather did high carbon dioxide concentrations tend to reduce the incidence of the disorder. However, the effect of carbon dioxide on Granny Smith apples, grown in Queensland and stored under controlled atmospheres, has been very significant. Scald incidence has been related to maturity and the injurious effects of carbon dioxide can be overcome by picking the fruit at a late stage of maturity. A similar trend had been noted in previous years. Neither the length of the storage period nor the age of the tree had any effect on the incidence of scald.

There was an increase in the amount of *total wastage* with increase in length of storage. Fruit from the second pick had less wastage than that from the first pick. For each pick the differences in total wastage in fruit stored under the different atmospheres were not significant. However, the controlled atmospheres increased superficial scald in the first pick but reduced soggy breakdown in both picks.

The *firmness* of the fruit decreased as the storage period lengthened. Fruit from both the controlled atmospheres was firmer than that from air storage. The increase in firmness with increase in carbon dioxide concentration was of the same order as in the 1954 experiments. Fruit stored in the 16 per cent. oxygen plus 5 per cent. carbon dioxide atmosphere was about $\frac{1}{2}$ lb. firmer than that stored in the 10 per cent. oxygen plus 2·5 per cent. carbon dioxide. Fruit stored in the higher carbon dioxide mixture was about 2 lb. firmer than the control fruit. Firmness was not affected by maturity or by tree age.

(c) Discussion.

The results indicate that picking maturity is the most important factor in controlled atmosphere storage. If fruit of correct maturity is stored, then increased scald due to high carbon dioxide concentrations is avoided and wastage from soggy breakdown is reduced. Further, such fruit is much firmer than if kept in normal air storage. Thus controlled atmosphere storage will give better results than normal air storage methods. Of the two atmospheres used, the mixture containing 16 per cent. oxygen plus 5 per cent. carbon dioxide resulted in slightly firmer fruit and less breakdown. Huelin and Tindale (1947)

found this atmosphere to be the most satisfactory of a number they tested with Victorian Granny Smith apples. This atmosphere is easier to maintain than the 10 per cent. oxygen plus 2·5 per cent. carbon dioxide mixture because it can be maintained by simple ventilation of the storage chamber.

The results are in good agreement with those obtained previously and supports the previous recommendation to use later picking dates.

(4) Effects on Keeping Quality of Delays Prior to Storage.

It is a common practice for some growers in southern States to hold their fruit for a period in the packing shed before placing it in store, as the delayed storage results in a reduction of scald, particularly in the early picks. Tindale and Huelin (1939) investigated the effects of delay on the incidence of superficial scald of Victorian Granny Smith apples held under refrigerated air storage and found that good control was effected with delays of 10–12 days provided the fruit was picked at the correct stage of maturity. However, if delays greater than this period were used there was a marked increase in the incidence of the disorder. The 1954 experiments showed a highly significant decrease in the incidence of superficial scald with increase in maturity. In view of this fact, the effects of delay after picking were studied, particularly in relation to controlled atmosphere storage.

(a) Experimental Details.

Fifty-four half-bushel cases of fruit were obtained from each of four growers; they comprised 27 cases from each of young and old trees. Only one pick was made—on Apr. 12, corresponding to Maturity 3.

Three storage atmospheres were used—normal air storage, 16 per cent. oxygen plus 5 per cent. carbon dioxide, and 10 per cent. oxygen plus 2·5 per cent. carbon dioxide. Three delay periods were investigated—nil, 7 days and 14 days. After picking, the fruit was wrapped in oiled wraps, packed into cases, and stored at the end of the delay period in the C.O.D. Cool Store, Thulimbah, in airtight cabinets, at 34-36 deg. F.

The atmospheres within the storage cabinets were allowed to build up to the approximate concentrations and then adjusted with nitrogen and carbon dioxide. During the storage period regular checks on the concentration of oxygen and carbon dioxide in the storage atmospheres were made with an Orsat gas analyser and any necessary adjustments made with nitrogen and carbon dioxide. Removals of fruit from the cabinets were made on Oct. 3 (Removal 1), Nov. 7 (Removal 2), and Nov. 28, 1955 (Removal 3).

After each removal was made, the atmospheres in the cabinets were re-adjusted to the required concentrations with nitrogen and carbon dioxide. The fruit, after removal from store, was railed to Brisbane, held at air temperatures for seven days to simulate normal marketing delays and then inspected for storage disorders.

(b) Results.

The results are shown in Table 6.

Table 6.

ADJUSTED MEAN PERCENTAGES OF DEFECTS, AND FIRMNESS, OF GRANNY SMITH APPLES AFTER REMOVAL FROM CONTROLLED ATMOSPHERE STORAGE—DELAYED STORAGE.

—	Mould.	Soggy Breakdown.	Superficial Scald.	Total Wastage.	Firmness.
No delay—					
Control	10·2	13·8	6·3	32·0	11·76
10% O_2; 2·5% CO_2	5·6	5·9	22·6	35·4	13·44
16% O_2; 5% CO_2	5·6	2·7	31·3	40·3	13·58
Removal 1 ..	8·3	0·5	15·6	25·4	13·67
Removal 2 ..	6·6	5·8	23·6	37·8	13·00
Removal 3 ..	6·1	16·7	21·1	45·1	12·11
Old trees	4·1	6·1	20·2	32·6	12·80
Young trees ..	10·0	8·8	19·8	39·2	13·06
7 Days' delay—					
Control	18·4	10·9	1·9	33·4	11·20
10% O_2; 2·5% CO_2	13·3	10·7	3·4	28·1	11·43
16% O_2; 5% CO_2	11·9	11·1	1·4	25·1	11·14
Removal 1 ..	22·6	1·0	2·5	26·2	11·79
Removal 2 ..	12·9	8·7	1·4	24·9	11·50
Removal 3 ..	7·2	24·1	2·8	35·7	10·48
Old trees	11·2	12·4	3·1	28·8	11·06
Young trees ..	17·7	9·5	1·4	28·9	11·46
14 Days' delay—					
Control	12·2	12·8	1·7	28·3	11·13
10% O_2; 2·5% CO_2	11·5	7·4	2·4	22·8	12·00
16% O_2; 5% CO_2	16·0	6·5	1·3	25·4	11·02
Removal 1 ..	15·1	0·5	1·3	18·4	12·29
Removal 2 ..	13·9	8·5	2·1	26·0	11·19
Removal 3 ..	10·6	18·3	2·0	32·6	10·67
Old trees	11·2	8·4	3·2	24·9	11·29
Young trees ..	15·2	9·4	0·4	26·0	11·47

Mould: No delay sig. less than 7 days and 14 days (1% level).
Removal 3 sig. less than Removal 2 (1% level).
Removal 2 sig. less than Removal 1 (5% level).
Old trees sig. less than Young trees (1% level).
No sig. diff. in Removals for No delay.
Removal 3 sig. less than Removal 2 and 1 (1% level) for 7 days.
Removal 2 sig. less than Removal 1 (1% level) for 7 days.
Removal 3 sig. less than Removal 1 (1% level) for 14 days.
Removal 3 sig. less than Removal 2 (5% level) for 14 days.

Total Wastage: 14 days sig. less than No delay (1% level).
Removal 1 sig. less than Removal 3 (1% level).
Removal 2 sig. less than Removal 3 (5% level).

Firmness: No delay sig. greater than 7 days and 14 days (1% level).
10/2·5 sig. greater than 16/5 (5% level).
16/5 sig. greater than Control (1% level).
Removal 1 sig. greater than Removal 2 (1% level).
Removal 2 sig. greater than Removal 3 (1% level).
Young trees sig. greater than Old trees (5% level).
No. sig. diff. in atmospheres for 7 days.

There was an increase in *mould* wastage with a delay of seven days, but wastage did not increase significantly beyond that point. Wastage was not affected by the storage atmospheres, but fruit from old trees had less mould than that from young trees. When there was no delay there was no difference between removals, but with delayed fruit the increase in wastage was marked as the length of the storage period increased.

No analysis of *soggy breakdown* was made, as 60 per cent. of that present was found in fruit from one of the four growers whose fruit was used in the experiment. Some trends do appear, however, and are worthy of mention. In the no-delay fruit, there was less breakdown in the 16 per cent. oxygen plus 5 per cent. carbon dioxide atmosphere than in either the 10 per cent. oxygen plus 2·5 per cent. carbon dioxide atmosphere or the normal air storage control. When the fruit was delayed, differences were not apparent. There did not appear to be any difference in wastage between fruit from old trees and that from young trees. Irrespective of the delay period, there was a considerable increase in soggy breakdown with increase in the length of storage.

Appreciable wastage from *superficial scald* occurred only in the no-delay fruit. Delays considerably reduced scald irrespective of the storage atmosphere. Neither age of tree nor time of storage affected the incidence of the disorder.

Storage atmosphere and tree age had no influence on *total wastage.* Fruit from the 14 days' delay had less wastage than that placed in store immediately after picking, but this was due to the large amount of scald present in the no-delay treatment. The effect of length of storage was significant.

Fruit from the no-delay treatment was of greater firmness than that which was delayed. In addition, fruit from the controlled atmosphere storage was firmer than that from normal air storage when there was no delay. Fruit from young trees was firmer than that from old trees, but the difference was of the order of only $\frac{1}{2}$ lb. The fruit softened with increase in the storage period.

(c) Discussion.

The results show that the incidence of superficial scald can be reduced to a minimum by delaying storage, irrespective of the atmosphere in which the fruit is stored. However, delay results in a reduction of firmness by at least 1 lb. and the fruit is also much more susceptible to mould and soggy breakdown. Thus, delayed storage cannot be recommended, since better control of storage disorders can be obtained by delaying the picking of the fruit

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VALUE OF 2,4,5-TP AS A PRE-HARVEST DROP SPRAY IN APPLES AT STANTHORPE

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SUMMARY.

Trials were carried out with 2,4,5–TP as a pre-harvest drop spray on Gravenstein apples.

When 2,4,5–TP was applied at a concentration of 50 p.p.m. at or shortly before the commencement of natural fruit fall, pre-harvest drop was reduced to negligible proportions (8.7 per cent. as against 25.7 per cent. in the case of ANA at the standard concentration of 10 p.p.m.). Observational data suggest that the concentration of 2,4,5–TP could be substantially reduced, if necessary, but such reduction is normally unjustified in view of the variable tree vigour in commercial orchards and other factors.

The efficiency of 2,4,5–TP as a pre-harvest drop spray can be largely attributed to its long period of effectiveness (26 days as against 14 days in the case of ANA) and the consequent increased permissible latitude in timing.

I. INTRODUCTION.

For some years past, alpha-napthalene acetic acid (ANA) has been widely used as a pre-harvest drop spray in apple orchards at Stanthorpe on early-maturing varieties such as Gravenstein, Jonathan and Delicious, which are very prone to premature fruit fall.

As in other countries, the results obtained in commercial practice have been highly variable, ranging from very good to very bad. Such variability has been ascribed to a number of causes, chief among which are :—

(1) *Faulty timing.*—Normally the spray is applied just before fruit drop is expected to occur but the precise date varies according to seasonal conditions and is not always easily determined.

(2) *Limited period of effectiveness.*—If treatment ceases to be effective before the fruit is fully mature, fruit drop may be accentuated.

(3) *Vigour of the trees.*—Response to ANA treatment is reduced in trees which are lacking in vigour owing to nutrient deficiencies, inadequate soil moisture or disease infection. Double treatment at short intervals is not a complete solution to the problem.

The possibility of other hormones being of greater value than ANA for the prevention of pre-harvest drop in apples has recently attracted attention. 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) was one of the first materials tested; it showed considerable promise at concentrations between 25 and 75 p.p.m. (Hatton 1955). A closely related product, 2,4,5-TP (2,4,5-trichlorophenoxy propionic acid), gave just as good a result as 2,4,5-T in a wide range of varieties.

II. PRELIMINARY OBSERVATIONS—1953-54.

In 1954, limited stocks of 2,4,5-TP became available for experimental purposes and an observational trial was established at Thulimbah on 16-year-old trees of the early-maturing variety Gravenstein.

Treatments were:—(1) ANA (10 p.p.m.); (2) 2,4,5-TP (10 p.p.m. prepared from a concentrated solution in methyl alcohol); (3) 2,4,5-TP (20 p.p.m. prepared from a concentrated solution in methyl alcohol); (4) 2,4,5-TP (20 p.p.m. prepared direct in water); and (5) control.

Sprays were applied through a knapsack pump on Jan. 4, 1955, and harvesting took place between Jan. 17 and Jan. 24.

The percentage fruit drop (means of duplicate trees) recorded were as follows:—ANA, 39·3; 2,4,5-TP (10 p.p.m.), 4·2; 2,4,5-TP (20 p.p.m. methyl alcohol), 5·1; 2,4,5-TP (20 p.p.m. water), 4·5; control, 16·9.

From these preliminary observations, there seemed little doubt that 2,4,5-TP warranted further investigations as a pre-harvest drop spray. The relatively high fruit drop in trees treated with ANA in this trial (39·3 per cent.) is noteworthy as an example of the hazards associated with its use in years when harvesting is preceded by hot, dry weather.

Table 1.

Climatic Data for Stanthorpe (1953–1955).

Rainfall (points).

June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Season.
3	37	185	6	158	270	46	124	1,193	115	91	90	1953–54
185	291	221	230	746	425	322	98	243	311	256	331	1954–55
189	199	169	220	251	275	349	372	332	269	161	179	Average
Temperatures—Mean Maximum (°F.).												
59·1	57·3	56·8	62·0	68·4	75·7	86·0	74·6	75·2	78·0	78·3	63·6	1953–54
57·6	59·0	61·2	63·0	68·7	74·7	76·3	80·0	79·5	76·1	70·6	60·3	1954–55
Temperatures—Mean Minimum (°F.).												
31·7	28·2	33·8	38·8	47·4	52·8	58·0	59·7	62·5	56·7	50·0	40·1	1953–54
36·6	38·0	38·5	41·4	48·9	54·0	57·5	59·5	61·5	63·7	53·5	42·5	1954–55

III. REPLICATED TRIAL—1954-55.

Following the observational trial in 1953-54, a replicated field trial was established in the same orchard at Thulimbah in 1954-55 on Gravenstein apples. Treatments were:—

(1) ANA (10 p.p.m.) applied Dec. 23 at the commencement of fruit drop.

(2) ANA (10 p.p.m.) applied Dec. 23 and again on Jan. 6.

(3) 2,4,5-TP (50 p.p.m.) applied Dec. 23.

(4) 2,4,5-TP (50 p.p.m.) applied Jan. 6.

The trial was established as a 6 x 4 randomised block with single tree plots. Treatment with ANA was withheld from five trees outside the experimental series in order to get an estimate of fruit drop under natural conditions.

Sprays were applied through high volume delivery equipment at a pressure of 400 lb. per sq. in. and at a rate of approximately 1·5 gal. per tree.

Unlike the previous year, soil moisture supplies in 1954-55 were adequate throughout the whole growing period (Table 1) and the trees at no time showed signs of stress.

Fruit falling to the ground under the experimental trees was collected daily from the time of the initial spray treatment on Dec. 23 until the end of the harvesting period. Progress fruit-drop totals on Jan. 6, Jan. 18 and at the close of harvesting are presented in Table 2 as percentages of the total crop.

Table 2.

FRUIT DROP (PERCENTAGE EQUIVALENT MEAN VALUES).

Treatment.	Jan. 6.	Jan. 18.	Total.	Mean Number of Fruit per Tree.
(A) ANA (single spray)	5·3	21·6	25·7	673
(B) ANA (double spray)	4·2	11·0	13·6	
(C) 2, 4, 5–TP (Dec. 23)	2·7	6·3	8·7	
(D) 2, 4, 5–TP (Jan. 6)	7·1	8·2	10·2	
	C < B, D B < D	C < B, A D, B < A	C < B, A D, B < B	
Control	10·2	30·5	32·5	

IV. DISCUSSION.

(1) Fruit Fall and the Season.

The incidence of natural pre-harvest drop is apparently highly variable from season to season—16·9 per cent. in 1953-54 and 32·5 per cent. in 1954-55. In the former year, rains in the early summer and midsummer periods were below normal (Table 1), the crop was light (Table 3) and the bulk of the fruit remained on the trees to maturity. In 1954-55, however, when fruit fall was heavy, soil moisture was ample throughout the whole drying period. It is inferred, therefore, that variations in soil moisture content are less conducive to fruit fall than the size of the crop and nutritional deficiencies when the fruit is approaching maturity.

Table 3.

YIELDS PER TREE AND PERCENTAGE FRUIT FALL.

—	1953–54.	1954–55.
Range	2–3 bus.	4–6 bus.
Mean	2·5 bus.	5 bus.
Fruit drop (%)	16·9	32·5

The relatively heavy fruit crop in 1954-55 may have been due to the effect of (*a*) excessive transpiration during the later stages of fruit development when the uptake of moisture from the soil was reduced by waterlogging; and/or (*b*) insufficient nutrient reserves, following leaching after heavy rains earlier in the year, to mature the developing fruit.

(2) Time of Application.

It is normal practice to apply pre-harvest drop sprays just before the onset of fruit drop, which usually begins some 10-12 days before the anticipated time of the first harvest. In the Gravenstein apple, harvesting usually begins in early to mid-January and sprays were therefore applied on Dec. 23. Substantial fruit drop occurred in the orchard by Jan. 6 and increased sharply during the next fortnight in all trees other than those receiving 2,4,5-TP. The failure of the ANA single-spray schedule and the moderate performance of the double-spray schedule suggest that the initial treatment applied on Dec. 23 was too early or alternatively that, under the conditions of this experiment, ANA applied at any time would have been ineffective. Certainly, the initial application had lost its efficiency shortly after Jan. 6—i.e., 14 days after treatment. The second spray applied on Jan. 6 reduced the amount of fruit drop but did not eliminate it.

The most effective treatment schedule was a single spray of 2,4,5-TP applied on Dec. 23. It was more efficient than the ANA double-spray schedule (Dec. 23 and Jan. 6) and the single 2,4,5-TP spray on Jan. 6. The latter was clearly applied too late. It would, therefore, appear that 2,4,5-TP remained effective over a period of at least 26 days.

(3) ANA versus 2,4,5-TP.

Although ANA sprays have for long been used to prevent pre-harvest drop in some varieties of apple in Queensland, failures are rather common and a substitute has been needed for some time.

The experimental evidence presented here suggests that, other things being equal, 2,4,5-TP at 50 p.p.m. is more efficient and should be used in commercial practice. It retains its effectiveness over a long period and times

of application need not be so precise. The risk of a breakdown under diverse climatic conditions, variable tree vigour and other factors is therefore remote.

The concentration of 2,4,5-TP used in the 1954-55 trial (50 p.p.m.) is much greater than that used in the observational trial of the previous year. It represents a middle concentration based on investigations in the U.S.A. and may be much higher than is strictly necessary. However, the cost of materials is of negligible importance in the pre-harvest drop spray programme and there is therefore little point in establishing power limits of usefulness which must inevitably vary from orchard to orchard and from year to year.

(4) Other Effects.

2,4,5-T and 2,4,5-TP have both been credited with the ability to hasten fruit maturity and intensify skin colour in the fruit (Thompson 1952). No such changes were observed in this trial.

These sprays are also said to reduce the storage life of the fruit. This point is of little interest in the Granite Belt, as the varieties which are most prone to pre-harvest drop (i.e., Gravenstein and Jonathan) are both early-maturing types which are not normally stored for long periods.

V. CONCLUSIONS.

(1) 2,4,5-TP at 50 p.p.m. is much more efficient in controlling pre-harvest fruit drop in the Gravenstein apple than ANA at 10 p.p.m., which has been in commercial use for many years in the Stanthorpe district.

(2) The period of effectiveness of the 2,4,5-TP spray is at least 26 days, as against 14 days with the ANA spray. The additional period of effectiveness allows a greater margin of safety in timing spray applications. This is an advantage in years when difficulties arise in relating the time of treatment to the anticipated date of fruit maturity. The risk of lack of response or induced fruit fall just before normal maturity, both of which are recognised hazards with ANA, is therefore negligible.

(3) 2,4,5-TP had no obvious effect on the time of fruit maturity or the skin colour of the fruit.

(4) The percentage fruit fall in 1953-54, a relatively dry year, was considerably less than in 1954-55, when the soil was consistently near field capacity. In the latter year, fruit fall may have been accentuated by high transpiration in January when the soil was partly waterlogged or, alternatively, by a deficiency of plant nutrients in the soil following leaching earlier in the season.

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